INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY
SIOP XXVIII MEETING
VIENNA, AUSTRIA
OCTOBER 1-5, 1996

ABSTRACTS CONTENTS

ORAL PRESENTATIONS

*Refers to abstract number

745 abstracts were received by February 29, 1996 and were evaluated by the Scientific Committee. Further, there were abstracts accepted from symposium contributors and guest lecturers. The accepted abstracts are printed here in order of oral presentation (O-1 to O-292) and poster presentations (P-1 to P-310), followed by abstracts accepted for publication only (1 to 39).

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INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY SIOP XXVIII MEETING VIENNA, AUSTRIA

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ABSTRACTS

O-1

ACTIVATION OF THE CD95 (APO-1 / FAS) PATHWAY BY CYTOTOXIC DRUGS

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Anti-cancer drugs used in chemotherapy of leukemias and solid tumors induce apoptosis in target cells. The molecular mechanisms by which apoptosis is induced by these reagents are not understood. In lymphoid cells the CD95 (APO-1 / FAS) system is a key regulator of apoptosis. Elimination of peripheral T cells is mediated via CD95 receptor (CD95) / ligand (CD95-L) interaction involving fracticide, paracrine death and autocrine suicide upon T cell receptor (TCR) triggering. We demonstrate that apoptosis induced by cytotoxic drugs at therapeutical concentrations in leukemia cells may involve the CD95 system. We used the prototype acute T leukemia (T-ALL) cell line CEM and doxorubicine, a drug used in chemotherapy of lymphoid leukemias. Doxorubicine strongly induced CD95-L mRNA expression *in vitro* at concentrations relevant for therapy *in vivo*. In addition doxorubicine-induced apoptosis was blocked by inhibition of gene expression and protein synthesis and was inhibited by blocking $F(ab')_2$anti-APO-1 (anti-CD95) antibody fragments. Furthermore activation of ICE-like proteases that are involved in the CD95 apoptosis pathway was found following treatment of CEM cells with various cytotoxic drugs. CEM and Jurkat cells resistant to CD95-mediated apoptosis were also resistant to doxorubicine-induced apoptosis and to other cytotoxic drugs such as methotrexate. In addition doxorubicine-resistant cells were found to be resistant to CD95-mediated apoptosis and were cross resistant to other cytotoxic drugs. The finding that apoptosis induced by anti-cancer drugs may involve the CD95 system provides a new molecular insight into resistance and sensitivity towards chemotherapy in malignancies.

O-2

PHASE I-II TRIAL OF TAXOL ACCORDING TO Q4D REGIMEN IN PEDIATRIC PATIENT WITH RECURRENT SOLID TUMORS.

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Previous in vitro studies with taxol in neuroblastoma (Helson L., Proc. ASCO Ab. 370, 1993) suggested a 4 day fractionated brief infusional schedule (Q4D) would be more effective than the standard 24 hour infusion every 21 days (Q 21D).

Starting at 60mg/m^2/90 minutes Q4Dx3, 26 patients (pt) 2-16 years old: primary brain tumors (9); neuroblastoma (4); Pnet (1); rabdomyosarcoma (2); Wilm's Tumor (2); retinoblastoma (1); hepatocellular carcinoma (1) entered the study.

We treated 3 pts, escalating at 15 mg/m^2 at each dose level; up to date two patients were treated at the dose level of 120 mg/m^2/ as a 160 minutes infusion.

Low grade anemia, trombocytopenia, diarrhea, granulocytopenia, and cutaneous rash was observed. Hypercholesterolemia and hypertriglyceridemia were observed in 100% of the patients.

Two patients experimented itch after taxol infusion.

One patient had appendicitis, and 1 patient peripheral neuropathy. Clinical evaluation was performed after 3 courses (9 doses).

Of the 26 patients treated so far, 25 are evaluable for response and toxicity.

Results: symptomatic improvement was observed in 12/25 pts., minor responses in 4 /25 and partial responses in 2/25 pts; one patient with Ewing's Sarcoma had complete disappearance of multiple pulmonary metastases and 1 pt. with ependimoma is in stable disease 34 months after therapy.

The maximum tolerated dose is still to be derminated and the study is ongoing.

O-3

EFFECTIVENESS OF ORAL ADMINISTRATION OF IRINOTECAN (CPT-11) IN THE TREATMENT OF NEUROBLASTOMA

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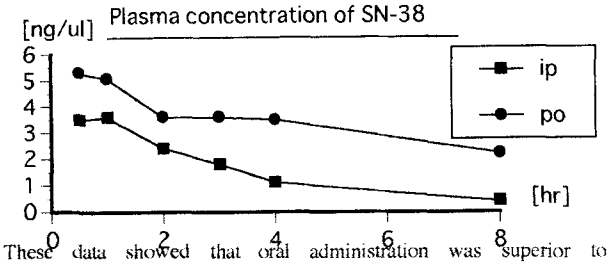
Although recent advances have been made in the management of neuroblastoma, this tumor remains one of the most challenging and frustrating malignant tumors. Little improvement has been achieved for patients in advanced stages, so the application of a more effective new drug has been awaited. We previously reported that CPT-11 was highly effective against human neuroblastoma xenograft TNB9. In this study, plasma pharmacokinetics of CPT-11 in different administration routes were evaluated. One third of LD50 was introduced either by intraperitoneally (59mg/kg) or by orally (404mg/kg) to BALB/c nude mice. Plasma concentration of CPT-11 and its active metabolite SN-38 in various time points were measured.



These data showed that oral administration was superior to intraperitoneal injection because plasma concentrations of SN-38 in oral administration were higher and these high level persisted longer. Oral administration of CPT-11 is highly recommended because it is a safer and better way in the treatment of high-risk neuroblastoma patients.

O-4

SAFETY AND EFFICACY OF AMIFOSTINE TO PREVENT THE MYELOTOXICITY OF HIGH DOSE CARBOPLATIN IN CHILDREN

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Amifostine (Ethyol) is a new chemoprotective agent. Here we report the preliminary results of the first phase I-II study with amifostine (AMI) and high dose carboplatin (HDC) conducted in children with relapsed and/or refractory malignancies. Our primary objective is to examine safety and tolerability of AMI in children, the secondary objectives include investigation of efficacy of AMI to prevent or to decrease the myelotoxicity of HDC. AMI has been given in a 15 min i.v. infusion at a dose of 740 mg/m^2 prior to and 2 hours following the administration of 600 mg/m^2 carboplatin on Day 1 and Day 2 of the treatment course, together with standard antiemetic therapy with ondansetron or tropisetron with dexamethasone. So far, seven patients entered the study, 3 with brain tumor, 1-1 with neuroblastoma, PNET, soft tissue sarcoma and Wilms tu., respectively. The median age of the pts is 12 years, the male-female ratio is 5:2. All patients have been previously heavily pretreated due to relapsed or refractory disease. For all 7 pts 28 infusions of AMI are evaluable for tolerability and safety assessment, and 6 pts are evaluable for efficacy. The following side effects attributable to the applied study regimen have been observed: >20 Hgmm decrease in systolic blood pressure in 5, >3 vomiting episodes in 8, nausea in 18, decrease of body temperature >1 °C in 3, subjective complains in 17, decrease of serum Ca in 4 and tetanic seizure in 1 of 28 infusions, respectively. All side effects have been reversible. Carboplatin related hematopoietic toxicity has been reduced in 4 of 6 evaluable patients, with a complete hematopoeitic recovery in 2 of 6 pts by day 21 following treatment, and a substantial reduction in bone marrow toxicity and in need for supportive therapy in 2 of 6 pts. No protective effect was evident in 2 of 6 pts. These preliminary results demonstrate that amifostine is safe and tolerable in children at this dose, and protective efficacy has been indicated in heavily pretreated patients. Further patient entry to this study is ongoing.

O-5

PHARMACOKINETICS OF ANTINEOPLASTIC DRUGS IN IN VITRO ASSAYS: POSSIBLE PITFALLS IN THEIR INTERPRETATION

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In vitro incubation assays are widely used for preclinical drug evaluation. In addition, in vitro toxicity assays have been established for the investigation of drug resistance profiles of individual leukemias and targeted therapy has been based on these results. Nevertheless, in vitro assays are arteficial systems and pharmacokinetics of the drugs in vitro is not normally monitored. We, therefore, investigated concentration versus time curves, metabolism, isomerization and effects on the culture media for several cytostatic drugs.
Methods: At various concentrations of **asparaginase** (asp), the enzyme activity as well as changes in asparagine, aspartic acid, glutamine and glutamic acid (HPLC) and the ammonia release were monitored in RPMI media for 24 hours. *The stability of activated* **oxazaphosphorines** (1.25-20 µg/ml **4-OOH-cyclophosphamide, 4-OOH-perhydroxyifosfamide and mafosfamide**) were monitored for 72 hours in RPMI (HPLC). Different **retinoic acid isomers (all-trans RA, 13-cis RA, and 9-cis RA)** were analyzed (HPLC) in RPMI (light protected) with/without 10%/20% FCS +/- cells. In vitro monitoring of Ara-C, etoposide, taxol and platinum is ongoing.
Results: Even with the lowest **asp** concentrations asparagine depletion was complete. Extreme, dose/time dependent changes in the amino acid profiles and a dramatic increase of ammonia were observed. All **oxazaphosphorines** showed a rapid decrease of in vitro activity down to about 10% within 4-6 hours and 2 % within 72 hours. The **retinoic acids** showed marked isomerization of about 20% during day 1 resulting in a complete equilibrium within 72 h (10-15% 13-cis, 20-25% all-trans and 65-75% 9-cis RA independent of the originally added isomer). In presence of HL-60 cells, the effect was less pronounced but still significant.
Conclusion: In vitro assays are not „stable" systems with fixed drug concentrations. Critical interpretation and the drawing of clinical conclusions requires the knowledge of what had happened in the cell culture system regarding drug concentration (oxazaphosphorines), isomerisation to additional toxic compounds (retinoids) and other pharmacodynamic effects (asparaginase). (Supported by BMBWi (# 01 EC 9401).

O-6

PROSPECTIVE PHARMACODYNAMIC STUDY OF HIGH-DOSE METHOTREXATE (HDMTX) IN OSTEOSARCOMA (OS) PATIENTS

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The histologic tumor response to preoperative chemotherapy (preopCT) is the best prognostic factor in OS patients treated by a HDMTX-based protocol. The inter-patient variability in MTX disposition may be responsible of treatment failures. To establish whether pharmacokinetically-guided dose adjustment may be relevant to improve the outcome of OS patients, we asked the following question: is there a relationship between systemic exposure (AUC) to MTX and tumor response? **Patients and Methods:** 33 OS patients (median age, 12 y) treated by the french national SFOP-OS87 protocol were prospectively studied during their preopCT in a single institution. 22 patients had a non metastatic OS, 9 a metastatic OS and 2 a radiation-induced OS. The preopCT included 7 HDMTX courses [weeks (wks) 1, 2, 3, 6, 7, 10, 11], 2 doxorubicin courses (70 mg/m^2, wks 4 and 8) and surgery on wk 12. HDMTX (8 g/m^2 in post-puberty patients, 12 g/m^2 in pre-puberty patients) was given over 4 h along with alcaline hyperdiuresis and folinic acid rescue. Blood samples were obtained over 72 h after each course (7 to 12 samples after courses 1 and 7, 4 after courses 2 to 6). MTX plasma levels were measured by an immuno-enzymatic assay (Abbott). Maximum likelyhood and bayesian estimations of individual pharmacokinetics (PK) were performed with the APIS programm (MIIPS, Marseille, France) using a 2 or 3 compartment model. Histologic tumor response was assessed according to the Huvos grading system. **Results**: 177 out of 201 administered HDMTX courses (88%) were available for PK analysis. *The maximum concentration (Cmax) ranged from 537 to 2120 µM (mean±SD,* 1128±358 µM). The mean clearance rate (Cl) was 68±20 ml/mn/m^2 and the AUC ranged from 1692 to 13767 µMxh. The mean AUC was significantly higher after a dose of 12 g/m^2 than after a dose of 8 g/m^2 (6729 ±1877 versus 4982 ±1758 µMxh; p<0.0001), while no difference was observed in Cl corrected for body surface area. The 22 patients with a non metastatic OS were included in *the pharmacodynamic study: 12 had a good histologic response* (<5% of viable cells) (good responders, GR); 8 had a poor response and 2 a clinically refractory disease (bad responders, BR). The mean individual AUC was 5815±867 and 6204±1436 µMxh in GR and BR patients, respectively (NS). No significant difference was observed in their mean Cmax (1076±175 *versus* 1224±328 µM). **Conclusions:** HDMTX dose adjustment according to pubertal status is not pharmacokinetically justified in OS patients. Tumor response is not related to MTX systemic exposure during the preopCT of the SFOP-OS87 protocol.

O-7

RED BLOOD CELL METHOTREXATE AND 6-THIOGUANINE NUCLEOTIDES FOR INDIVIDUAL MTX/6MP DOSAGE IN ALL

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During MTX/6MP therapy, cytotoxic MTX-polyglutamates and 6-thioguanine nucleotides accumulate intracellularly including in erythrocytes (E-MTX and E-6TGN), where steady-state levels are achieved after 5-8 weeks of an unchanged dose. E-MTX and E-6TGN are related to both myelotoxicity and remission duration[1]. To explore the clinical significance of blood counts, MTX/6MP doses, and E-MTX/6TGN, the ongoing NOPHO ALL-92 study randomizes children with ALL on MTX/6MP maintenance therapy to have dosage adjustments by either WBC (target: 1.5-3.5x10^9/l) or by a combination of WBC and E-MTX/6TGN (pharm.-group). Patients in the pharm.-group with E-MTX*6TGN below 1350 (nmol/mmol Hb)[2] are recommended to have the doses of MTX and 6MP adjusted by 20% increments until an E-MTX*6TGN at or above 1350 have been achieved or the WBC falls below 1.5x10^9/l. Between Jan.92 and Dec.95 424 patients (median age: 4.2y; M:F 228:196) have been randomised (209 in the pharm.-group) with as yet no significant difference in outcome. Overall 4-year pEFS: 0.89±0.04 (15 relapses, 1 secondary AML, 3 deaths in remission). Patients relapsing had lower E-6TGN and E-MTX*6TGN levels than patients staying in remission, but did not differ in respect to WBC-levels. Increased emphasis on the titration of MTX/6MP dosage seem to have added to the improvement in pEFS. Although titration of MTX/6MP-doses by E-MTX/6MP is feasible, further follow-up are needed to determine its independent impact on outcome.

[1] Schmiegelow et al. J Clin Oncol 1995;13:345-351

O-8

RANDOMIZED COMPARISON OF 6-MERCAPTOPURINE (MP) VERSUS 6-THIOGUANINE (TG) IN CHILDHOOD ALL: PHARMACOLOGY, HEMATOLOGIC TOXICITY, AND PRELIMINARY CLINICAL RESULTS

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Background: MP has been the standard drug in maintenance treatment of childhood ALL. MP as well as TG, the latter more directly, are converted to 6-thioguanine nucleotides (TGN). TGN levels, measured in red blood cells (RBC), have been shown to correlate with prognosis.

Methods: In the ongoing treatment study for childhood ALL, COALL-92, pts are randomized during maintenance treatment to receive either MP or TG p.o. daily as well as methotrexate (MTX) p.o. weekly. Drug doses are adjusted to the white blood count (WBC). Values for WBC, platelets, doses of MP, TG, MTX, and reasons for treatment interruptions are recorded centrally. In 54 patients 3 monthly measurements of TGN and the methylated derivates of MP and TG were performed with HPLC.

Results: 339 pts were entered into the study, 308/319 pts who reached maintenance treatment agreed to randomization. In 128 pts, who had received either MP or TG for a mean of 60 weeks, hematologic toxicity was analyzed. The mean dose of MP was 357 µMol/m² and the mean WBC 3.2/nl; the corresponding values for TG were 237 µMol/m² and 3.7/nl. Mean RBC-TGN levels were 6 times higher after TG (2035 vs. 341 pmol/10^8 RBC) whereas methylated (Me) metabolites were much higher in the MP group (MeMP: 11458 pmol/10^8 RBC) than in the TG group (MeTG: 981 pmol/10^8 RBC). MeTG after MP was found only very infrequently. Mean platelet count with TG was 177/nl vs. 233/nl with MP. A platelet count <100/nl was 10 times more frequent in the TG group. EFS after 3.5 years is 0.84 for MP and 0.85 for TG.

Conclusion: TG differs markedly from MP in its metabolic conversion as well as its hematologic toxicity. Similar leukocytopenia is achieved with two thirds of the MP dose and thrombocytopenia is frequent. A longer observation time is needed to show whether the 6 times higher TGN levels are of therapeutic advantage.

O-9

MELPHALAN PHARMACOKINETICS IN CHILDREN WITH SOLID TUMOURS.

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Melphalan and carboplatin are frequently used at high doses as part of megatherapy with marrow rescue for children and adults with a variety of malignancies. We have used a standardised regimen of carboplatin and melphalan in 25 children. Although the carboplatin dose is adjusted on renal function, melphalan is given at a standard dose of 180mg/m², with no modification for renal or other function. We studied 12 children who received 180mg/m² melphalan preceded by 5 days of carboplatin. All had melphalan pharmacokinetics studied after the 180mg/m² dose; 10 also had levels measured after a 10mg/m² "test" dose on the day before the carboplatin commenced. Four patients received total body irradiation in addition to the carboplatin. No patient developed renal impairment during the 5 days of carboplatin. Melphalan levels fitted a two compartment model. Unlike previous reports and despite the use of intervening chemotherapy and radiotherapy, we found that all pharmacokinetic parameters obtained with the test dose correlated with those of the full dose. For example, the AUC predicted from the test dose correlated positively with the AUC from the full dose ($r = 0.9$, $p < 0.0005$). The mean AUC after 180mg/m² was 19.4 (± 2.5) times that obtained after the 10mg/m² dose. This suggests it may be possible to predict the full dose characteristics based on the test dose. This will be clinically relevent if full dose melphalan pharmacokinetics correlate with toxicity post-BMT.

O-10

PHARMACOKINETIC AND PHARMACODYNAMIC MONITORING OF ASPARAGINASE TREATMENT AND SUBSEQUENT DOSE ADJUSTMENT

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L-asparaginase (Asp.) from different biological sources (Escherichia coli, Erwinia chrysanthemi) is prescribed to completely deplete asparagine in blood. Faced with increasing reports of treatment complications a program to monitor enzyme activity and asparagine levels in serum was started. Based on the monitoring data, then dose optimisation of the Asparaginase medac™ (currently the only available E.coli preparation in Germany) was established.

Part I: Trough levels of asp.acitivity (enzyme assay) and asparagine (HPLC, limit of det. 0.1 µM) were measured in 49 children on induction treatment (8 applications, 10000 U/m² every 3rd day) for childhood acute lymphatic leukemia and non-Hodgkin's lymphoma with different E. coli preparations (Asp. medac™, Crasnitin™) and in 52 children on reinduction (Asp. medac™, Crasnitin™, and, in the event of allergic reactions, Erwinase™).

During induction, both E. coli preparations led to the desired reduction in asparagine, however, asp. activity with Asp. medac™ was significantly higher than with Crasnitin™ (median trough levels 430 vs 74 U/l). Under reinduction (trough levels: Asp. medac™ 528 U/l, Crasnitin™ 49 U/l, Erwinase™ < 20 U/l) complete asparagine depletion was recorded in more than 90 % of Asp. medac™ samples, 64 % of Crasnitin™ samples and a mere 26 % of Erwinase™ samples.

Part II: Following pharmacokinetic simulation on the basis of these monitoring data, 10 children received induction therapy with only 5000 U/m² Asp. medac™. The median trough level was still 255 U/l. In a subsequent group of 15 children on 2500 U/m² Asp. medac™ the median trough level was 96 U/l. In both groups asparagine depletion was complete in plasma and cerebrospinal fluid.

Conclusions: Different asparaginase preparations are not readily interchangeable. When Crasnitin™ is replaced by Asp. medac™ during induction therapy, comparable enzyme acitvities will be reached with only 1/4 of the asp. dose. When substitution of an alternate source of asparaginase is neccessary, monitoring of the activity is advisable. Supported by BMBWi (# 01 EC 9401).

O-11

A MURINE MODEL FOR BONE AND BONE MARROW METASTASIS

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Several experimental models of metastasis for various kinds of malignancy have been presented, however, animal models for bone and bone marrow metastases are rarely reported. In this paper, a reproducible tumor model for bone and bone marrow is reported, which was developed by an injection of murine C-1300 neuroblastoma(NB) cells into the tail vein of syngeneic A/J mice. After cell injection, the animals died with liver metastasis, at 18-21days , and often had bone marrow metastasis in the femur. A maturational agent was administered to inhibit liver metastasis and to extend survival in mice with advancing bone metastasis. Histological examination of bone marrow metastasis demonstrated lesions varying from a few small colonies of C-1300 NB cells either in metaphysis or diaphysis to large foci replacing normal haematopoietic bone marrow, simultaneously invading epiphysis or cortex of bone as bone metastasis. This model demonstrated the ability to detect NB cells in bone marrow histologically and could determine TD50 by extraction of bone marrow cells after treatment with various doses of drug or irradiation. In vitro assay of bone and bone marrow tumor cells exhibited the characteristic subclone different from the other subcoles which originated from C-1300NB.

This experimental system allows for investigations into the therapeutic response and biology of NB metastasis in bone and bone marrow.

O-12

CYSTEINE PROTEINASES ARE INVOLVED IN OSTEOSARCOMA CELL INVASION *IN VITRO*.

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In order to invade normal tissues and metastasize, cancer cells have to degrade basement membranes. Proteinases are believed to be essential in this process. Osteosarcoma cells as well as normal osteoblasts are known to secrete plasminogen activators, but there is very little information concerning the involvement of cysteine proteinases in osteosarcoma cell invasion. We have studied the possible effects of two cysteine proteinase inhibitors (E-64 and leupeptin) on osteosarcoma cell invasion *in vitro*.

MATERIAL AND METHODS. The osteosarcoma cell line OHS was studied in an experimental cell invasion model. Filters (8µm pores) were coated with the solubilized tissue basement membrane Matrigel. After 48 h incubation, cells that had passed the basement membrane and filter and adhered to the lower part of the filter were counted. Possible effects on motility using uncoated filters, adhesion to Matrigel-coated wells and tumor cell growth were also studied.

RESULTS. Nontoxic concentrations of the proteinase inhibitors reduced the invasion of OHS cells through Matrigel, leupeptin by 30 % and E-64 by 33 %. None of the proteinase inhibitors reduced tumor cell motility through uncoated filters or adhesion to Matrigel-coated wells. Tumor cell growth were not affected by the proteinase inhibitors.

CONCLUSION. These results, using an experimental cell invasion model, show that cysteine proteinases are of importance for osteosarcoma cell invasion.

O-13

THE ANGIOGENESIS INHIBITOR TNP-470 REDUCES THE GROWTH RATE OF HUMAN NEUROBLASTOMA

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Neuroblastoma is a well vascularized tumor that often presents with metastases. Tumor growth and metastasis are considered to be angiogenesis dependent. Inhibition of angiogenesis could be a new therapeutical approach in neuroblastoma. The aims of this investigation were to develop an animal experimental model of neuroblastoma and to investigate the effect of the angiogenesis inhibitor TNP-470 on tumor growth. Twenty-three nude rats (WAG rnu/rnu) were used as hosts, and a poorly differentiated human neuroblastoma cell line (SH-SY5Y) was used as xenograft by s.c. injection in the rats. Tumor volume was measured with a caliper every other day. When the largest tumor in an animal had reached 0.3 ml, treatment with TNP-470 began, 10 mg/kg, given s.c. every other day. Eleven animals served as controls. Tumor take was 89% (41/46 injections in 23 rats). The tumors grew exponentially and reached 5.2± 1.6 ml two weeks after tumor take. No metastases were found. Tumor cells retained their phenotype at the ultrastructural level after transplantation, and expressed neuron-specific enolase and chromogranin A and B. TNP-470 gave a treated/control quotient for mean tumor volume of 0.34 after 12 days of treatment. Not only were treated tumors 66% smaller at autopsy, but they also exhibited larger areas of necrosis, apoptosis and hemorrhage. This is the first report of a reduced neuroblastoma growth during administration of an angiogenesis inhibitor. Angiogenesis inhibitors may become a less toxic complement to chemotherapy. Also, a new animal experimental model for human neuroblastoma is defined.

O-14

PRIMARY BONE LYMPHOMA. SFOP experience (French Society of Pediatric Oncology).

Patte C, Brugière L, Bergeron C, Leverger G, Demeocq F, Berendt H, Lejard O, on behalf of the SFOP.

Data of the SFOP lymphoma studies since 1989 were reviewed relating to primary bone tumors. Lymphoma arising in maxillaries were excluded. Among 678 study entered patients (pts), 20 (3 %) had primary bone lymphoma. 10 were girls and 10 boys. Age was from 3 to 17 years, median 11. Disease was revealed by pain ± tumor. Histo-immunology was : 6 diffuse large B cell (L B C), 5 Burkitt's (Bu), 5 precursor B lymphoblastic (Lb), 1 peripheral T cell (PTC), 3 anaplastic large cell CD30 + (ALC). Primary tumor sites were : 5 femur, 5 tibia, 3 humerus, 3 iliac bone, 2 vertebra, 1 scapula, 1 sacrum. Lesion was lytic on X-Ray, often involving epiphysis. Extension outside of the bone locally and at distance was usually correlated with Bu, Lb or ALC. 9 pts had only one involved bone, 5 pts had two or more bones and 6 pts had other tumoral sites : regional nodes (3), gonade (2), spleen (1) pancreas (1). There were 7 stages I, 4st II, 7st III, 2 st 4 (nerve infiltrations, blasts in CSF). None had marrow involvement on cytology smears.

14 pts were treated with the LMB 89 protocol for B cell lymphoma, 3 with the LMT 89 protocol for non B cell and 3 with the HM 89 and 91 protocols for ALC. Complete remission (CR) was difficult to evaluate because of slow bone reconstruction and persistance of bone abnormalities ; 9 pts had biopsies to confirm CR. 1 pt is too early for evaluation. 1 pt with st III Bu had histologically confirmed PR and had protocolar HD chemotherapy with ABMT. 3 pts relapsed : 1st III Bu at 5m (who died), 1st I LBC at 32m, both in regional node, 1st III Lb at 18m in bone + BM. With a median follow up of 39m (75-6), 18/19 evaluable pts are alive, 2 of them in 2nd CR. None received local RTH and 1 had a tumor resection.

In conclusion : Bone lymphoma are rare (3 % of all lymphoma). Many histo-immunological types can be seen. Outcome is similar to the one of lymphoma of other sites and histology. RTH is not needed for local control.

O-15

BONE DISEASE IN LANGERHANS CELL HISTIOCYTOSIS (LCH). Results from the DAL HX-83 and 90 Studies.

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In 2 consecutive prospective multicentric clinical trials DAL HX-83 and 90 267 patients (pts) with newly diagnosed LCH and age <18 years were registered. 155 (58%) patients had single system bone disease; 121 (45%) had a unifocal bone lesion (UFB), 34 (13%) had multifocal disease (MFB).There was a predominance of boys (90 m, 65 f). The median age at onset was 6y 10m (range 8m -17y11m). Presenting symptoms were pain (65%), local swelling (51%), motion deficit (9%), pathological fracture (2%), neurological problems (4%). At diagnosis the pattern of lesions was equal in UFB and MFB: 40% of the lesions were found in the skull, 18% in the spine, 17% in the lower extremities, 10% in the pelvis, 8% in the upper extremities, and 7% in the thoracic bones. X-ray was superior to bone scan in the detection of lesions, especially in the spine (100% versus 74%) and pelvis (96% versus 72%);18% of lesions detected by x-ray were missed by bone scan. In UFB local treatment comprised surgery (79%), irradiation (23%), intralesional steroids (8%) or a combination of these modalities (18%), and systemic treatment included steroids (4%) or vinblastine (3%). MFB pts were treated with polychemotherapy including prednisone, vinblastine, etoposide and 6-mercaptopurine for one year. 146/155 pts (94%) showed a resolution or clear regression of the lesions after initial treatment, 2 pts responded after a change of therapy and 7 patients (5%) experienced a progression of the disease. Reactivations were observed in 18% of pts both in UFB (22/121) and in MFB (6/34), which were multifocal bone lesions in 67% and 73%, respectively.The probability of remaining free of disease was 82% in UFB and MFB. At reactivation there was a predominance of skull lesions in MFB(88%), whereas in UFB the distribution of lesions was similar to that at diagnosis. At the time of evaluation 154/155 pts were free of disease. Permanent consequences linked to sites of disease were observed in 21% of UFB and 41% of MFB. They comprised orthopedic disabilities (49%), neurological problems (12%), tooth loss (19%), hearing impairment (8%) and diabetes insipidus (8%). In conclusion, the course and the outcome of LCH confined to bones is excellent and seems to be independent from initial therapy.

O-16

RENAL TUMOURS OF CHILDHOOD DEVELOPING BONE METASTASIS

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Our aim was to evaluate the histologic spectrum and the outcome for children with renal tumours developing bone metastasis in order to see whether there had been an improvement over time for the patients fate.

Material and methods: We analysed the renal tumours metastasizing primarily or later to the bone in the SIOP 1,2,5,6&9 trials and studies diagnosed between 1971 and1993.

Results:There were 59(1.9%) of 3065 primary renal tumours which developed bone metastasis including 39 of totally 81 clear cell sarcomas of the kidney(CCSK),17 Wilms tumours(WT) and 2 of totally 22 rhabdoid tumours of the kidney(RTK).Half of the CCSK had skeletal lesions at diagnosis(stage IV).In the WT group 3 showed anaplasia and many showed an extensive rhabdomyomatous component,probably due to the preoperative treatment.The WT metastasised and infiltrated locally the bone preferably at the time of relaps with still a chance to survive.The 2 RTK developed bone metastasis in primary progression of disease within 7 months after diagnosis and died without sign of remission.Although the prognosis as a 3-year event-free survival has improved since SIOP 1 to about 75 % in localised CCSK in SIOP 9,the three children with primary bone metastasis died within 13 months after diagnosis.In contrast, the chance for a progression-free survival in RTK with or without bone metastasis was still low as ever.

Conclusion:Primary renal tumours developing bone metastasis are a heterogenous group consisting of CCSK,WT and RTK.The presence of bone metastasis is a very bad prognostic sign and these patients require more intensive treatment.

O-17

TIME-AND DOSE-DEPENDENT UPREGULATION OF TNF alpha AFTER IRRADIATION OF EWING`S TUMOR CELLS IN VITRO AND IN VIVO

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Introduction: Radiotherapy plays a major role in the local treatment of Ewing`s sarcoma. Little is known about the molecular effect of irradiation in this tumor. In this study we determined the TNF alpha production of Ewing`s tumor cells in vitro and in vivo.
Materials and methods: A dose range of 2-40 Gy was given and the tumors were examined 1-72 hours after irradiation. Time- and dose-correlation of TNF alpha-mRNA and protein was performed for six different cell lines in vitro and two cell lines in vivo using the nude mouse xenograft model. TNF alpha messenger RNA was detected by quantitative RT-PCR and protein expression was determined by EASIA and immunhistochemistry.
Results: All 6 cell lines had a TNF alpha expression on mRNA level and 4 a protein production of different amount. 5 of the 6 cell lines showed an upregulation of TNF alpha mRNA and 4 of the protein up to sixfold of the basic expression. For 5 of the cell lines we found a time- and dose dependency of both mRNA and protein level. The same phenomenon could be seen regarding the tumors on the nude mice.
Conclusion: Ionizing irradiation leeds to a time- and dose-dependent increase of TNF alpha on the mRNA- and protein level in some Ewing`s tumor cell lines. The induction of cellular cytokine release may be an important mechanism of the biological effect of ioniszing radiation on Ewing`s sarcoma.

O-18

TARGETED INTERNAL RADIOTHERAPY IN OSTEOSARCOMA PATIENTS USING ^{153}Sm-EDTMP

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Approximately 40% of patients with osteosarcomas (OS) still succumb to the disease, mainly due to chemotherapy resistant metastases in the lungs, despite the improvements in aggressive combination chemotherapy. Tumors arising in the axial skeleton carry a dismal prognosis since the current multimodality treatment often fails to "sterilize" the local disease. Importantly, a majority of OS, including metastases, produce primitive bone matrix, giving intense "hot spots" on conventional diagnostic bone scans. Hence, we have explored the use of bone seeking radiopharmaceuticals, such as ^{153}Sm-EDTMP, as a new treatment modality in relapsing OS-patients with osteoblastic lesions. Ten patients with OS, presenting with either locally recurrent or metastatic disease after all conventional treatment modalities had failed, were subjected to ^{153}Sm-EDTMP treatment consisting in i.v. infusions of 25-60MBq/kg body weight on one to three occasions, usually six weeks apart when hematological parameters were restored. All patients had been heavily pretreated with chemotherapy. In all three patients where pain was present before treatment, the pain was significantly relieved. In one patient, bedridden with paraparesis and impaired bladder function due to a vertebral lesion, the pareses gradually subsided after treatment. Six months after the last radionuclide treatment the patient was without objective neurological symptoms, and without detectable metastases. Subsequently he experienced increasing pain. He became dependent on epidural morphine administration and died 2 months later from purulent meningitis. In five other patients significant growth delays were observed lasting for up to 18 months. One patient showed progressive tumor growth despite good uptake of the radiopharmaceutical in numerous metastatic lesions. Three other had relatively low tumor uptake and progressed rapidly. The absorbed radiation doses were calculated using attenuation-corrected conjugated view techniques by combined use of CT-information and whole body scanning with a dual head gamma camera. Calculations indicate that the dose levels may reach 80 Gy following a single course of the treatment. In these patients the treatment resulted in significant growth delay of their metastases. The effective halflife of ^{153}Sm-EDTMP in OS-lesions was equal to the physical halflife of ^{153}Sm. We conclude that targeted radionuclide therapy using ^{153}Sm-EDTMP may give considerable palliative effect in patients with osteoblastic osteosarcoma. An attractive treatment strategy, which is now beeing explored, is to use, already in the primary treatment of osteoblastic OS, ^{153}Sm-EDTMP as a boost technique to conventional external radiotherapy and as an adjunct to chemotherapy.

O-19

CISPLATINUM-INDUCED RESISTANCE TO RADIOTHERAPY IN HUMAN GLIOMA CELLS.

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The treatment of brain tumors includes surgery, chemotherapy and radiotherapy. There is a discussion, whether chemotherapy reduces the efficacy of radiotherapy, and which treatment should be given first.

We examined whether cisplatinum (CDDP) induces resistance against radiation in A172 human glioblastoma cells. The cells were pretreated with CDDP (10^{-6} mol/l) for 24h, surviving cells were cultured for 7 to 14 days, until they recovered to the original doubling time of 24 hours. This procedure was performed five times. Afterwards CDDP-pretreated and control cells were irradiated with 9 Gy using ^{60}Co gamma irradiation. 192h later surviving cells were measured with a colorimetric test system (MTT, 3.(4.5-dimethylthiazol-2-yl)2.5-diphenyl-tetrazolium bromide). 192h after irradiation only 17,8% of the control cells survived, while 25,1% of the cells pretreated with CDDP survived (n=8, p=0.0142). The experiment was replicated with the same results. These data show that cisplatinum-treatment can induce resistance against radiation in human glioma cells. However, more detailed experiments are necessary before this information can be transformed into clinical treatment protocols.

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O-20

PARTICLE BEAM THERAPY FOR PEDIATRIC MALIGNANCY

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Heavy ion radiotherapy possesses optimal properties for therapy, that is, improved beam localization by the use of the Bragg curve and biological advantages due to as increased density of local high-LET depositions. At the National Institute of Radiological Sciences (NIRS), the HIMAC(Heavy-Ion Medical Accelerator in Chiba)project was practically started in 1993. In this paper, the experimantal aspects of the heavy ion beam therapy against pediatric solid malignancy discussed. Using in vivo tumor intake assay, relative biological effects(RBE) between r-ray and carbon beam were investigated. Single cells of C-1300 murine *neuroblastoma* were injected into tail vein of female A/J mice to produce bone marrow metastasis.When tumorigenisity of bone marrow extract containing tumor cells examined, irradiation of 290MeV/u carbon units at a grading dose of 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 10, 12.5, and15Gy inhibited the development of new coming tumors in dose-depending fashion. RBE was 2.5-4.0. Heavy ion radiotherapy would be a great candidate as a new strategy.

O-21

BONE CANCER AFTER CHILDHOOD CANCER

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Individuals who had cancer in childhood are at higher risk of developing bone cancer than any other type of second primary cancer. Using the population-based National Registry of Childhood Tumours in Britain, we investigated the incidence and etiology of second primary bone cancer after childhood cancer in a cohort study and in a case-control study. A cohort study of 13,175 three-year survivors of childhood cancer diagnosed in Britain between 1940 and 1983 revealed 55 subsequent bone cancers. A largely nested case-control study comprised 59 case subjects developing second primary bone cancer, and 220 control subjects were selected and matched for sex, type of first cancer, age at first cancer, and interval between diagnosis of first cancer and subsequent bone cancer. The percentage of 3-year survivors developing bone cancer within 20 years did not exceed 0.9%, except following heritable retinoblastoma (7.2%), Ewing's sarcoma (5.4%), and other malignant bone tumours (2.4%). The risk of bone cancer increased substantially with increased cumulative dose of radiation to the bone ($p<.001$, linear trend). At the highest levels of exposure, however, the risk appeared to decline somewhat ($p = 0.065$, nonlinearity). Exposure to less than 10 Gy was, at worst, associated with only a small increased relative risk (RR) of bone cancer (RR = 0.7; 95% confidence interval = 0.2-2.2). The risk of bone cancer increased linearly ($p = .04$, one-tailed test) with increased cumulative dose of alkylating agents. This population-based study provides grounds for reassurance of the majority of survivors in that their risk of developing bone cancer within 20 years of 3-year survival did not exceed 0.9%. The higher risks found for bone cancer following the other specific rare types of childhood cancer provide a rational basis for surveillance. These risks should help in making decisions concerning future treatment protocols.

O-22

CHONDROSARCOMAS (CHSAs) CAN DEVELOP IN POST-IRRADIATION OSTEOCHONDROMAS (OSCHs)

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Generally considered to be of little clinical significance, OSCH is a relatively common benign new growth after radiation therapy (RT) given to children. It was present in 19 of 680 (3%) Wilms tumor (WT) long-term survivors reported by Evans et al.; (Cancer 67:331, 1991); 17/19 were in RT ports. Two CHSAs were included among 43 second malignant neoplasms (SMNs) in 5,278 WT patients followed for 4-25 yrs by Breslow et al. (J Clin Oncol 13:1851, 1995). Important is the fact that both these CHSAs evolved in post-RT OSCHs, one 21.2 yrs after 40 Gy to the pubis of a 4.3 year old boy, and 9 yrs after the OSCH was found; the other 16 yrs after 24 Gy to the ilium of a 2.8 yr old girl, 8 yrs after the OSCH appeared. Both patients are alive and free of disease 3 yrs after CHSA excision. Malignant changes in post-RT OSCHs are extremely rare, there being only one other example in the literature (Perez et al., Radiology 88:750, 1967). Conclusion: Malignant degeneration can occur in post RT OSCHs. They require careful and prolonged follow-up. Supported in part by NIH grants RO1 CA54498 and UO1 CA42326.

O-23

RADIATION-RELATED BONE SARCOMA : A RETROSPECTIVE STUDY OF 24 CASES

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Radiation therapy expose to a risk of secondary osteosarcoma. However, the prognosis of these second malignancies since the advent of intensive chemotherapy has seldom been evaluated. We report a retrospective study of 24 patients treated for a solid tumor during childhood and who developed a radiation related osteosarcoma between January 1981 and January 1996. Because of RB gene abnormality, patients with retinoblastoma as primary tumor were excluded from our study.

Patients : 18 males, 6 females, 3 patients had familial history of an excessive aggregation of cancer, 1 had fibro-osseous dysplasia, 1 had neurofibromatosis. Median age at the time of first tumor : 6.5 years (1-21).

First tumor : Ewing's sarcoma (9), rhabdomyosarcoma (4), malignant mesenchymoma (1), Hodgkin's disease (3), medulloblastoma (3), nephroblastoma (1), neuroblastoma (1), optic glioma (1), epithelioma (1). All patients were treated with radiotherapy, median dose : 45 grays (25-60). 20 patients received chemotherapy, including high dose alkylating agent for 19 of them.

Radiation related osteosarcoma : All patients developed an osteogenic sarcoma in the radiation field, histologically confirmed for all of them, with various differenciation. Median time between radiotherapy and diagnosis was 8 years (3.5 - 26). Localization were : fibula (1), tibia (1), femur (4), iliac bone (2), radius (1), humerus (2), omoplate (2), vertebra (1), rib (2), collar bone (2), mandibule (2), orbit (1), occiput (2), malar bone (1).

21 had localized disease, 1 had lymph node involvement, 1 had bone metastases, 1 had bone and lung metastases. 7 patients received palliative treatment (various chemotherapy regimen for all, with incomplete surgical resection for 1). 3 patients were treated by complete surgical resection alone. 14 patients received intensive chemotherapy containing HD MTX for 11, without HD MTX for 3, before and/or after complete surgical resection.

Follow-up : 17 patients achieved complete remission. 13 of them remained disease free with a median follow up of 5 years (5 months-10 years). 4 relapsed : local (1), bone (1), lung (1), bone and lung (1), and died from 18 months to 7 years after the diagnosis. 7 patients did not achieve complete remission : 6 died from 4 months to 3 years after the diagnosis, 1 is alive with disease 9 months after the diagnosis.

Conclusion : This report demonstrate that survival of patients with radiation related osteosarcoma is encouraging. Patients with resectable lesion should benefit of intensive pre and post operative chemotherapy.

O-24

SECOND PRIMARY TUMORS AMONG CHILDHOOD CANCER SURVIVORS. AN UPSET OF THE S.E.O.P. STUDY. (1969 - 1995)

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The Spanish Late Effects Study Group ,established in Spain in 1993 collects information on children treated for a previous cancer. Second malignant tumors (SMT) diagnosed before December 31, 1995 were identified trrough a special enquiry to the 40 institutions cooperating with S.E.O.P. Out of 10,750 study subjects **a total of 109 SMT** have been collected among **21 pediatric oncology centers** of Spain .Those patients were less than 18 years of age at diagnosis of first primary tumour (FPT) and were treated at those hospitals between 1969 and 1995. This represents one of the largest data base available for patient data for this important aspect of late effects of childhood cancer. These cases do not represent complete reporting of all SMT but includes patients whose initial diagnoses were made over twenty five years from twenty one major centers for pediatric cancer in Spain.The purpose of this report is to present a summary of the data that has been collected and to present an overview of the nature of the primary and subsequent cancers.

The most common FPT were acute lymphoblastic leukemia [ALL], non-Hodgkin lymphoma,CNS tumors ,retinoblastoma and soft tissue sarcoma. Median and range follow-up duration after first FPT was 6.6 years and 1-19 years. The Standard Incidence Ratio (SIR) is 13.8,Confidence Interval (C.I.) 95% :10-16. 109 patients developed a total of 109 SPT. **The most common SPT was acute nonlymphoblastic leukemia** [ANLL], CNS tumors,bone malignant tumors ,soft tissue sarcoma and carcinomas .

Direct comparisons with similar studies are therefore somewhat problematic. Our date suggest that risk of SMT in childhood ALL cancer survivors may be greater than previously reported ; CNS tumors are the most common SMT in this group and the higher risk of SMT comes from previous brain tumors. Occurrence of ANLL as SPT in children treated for a primary ALL was very high and confirms the potential risk for secondary ANLL in children with ALL who received a chemotherapy regimen which included an epipodophyllotoxin.

O-25

SECOND MALIGNANT NEOPLASMS (SMN) AFTER PRIMARY OSTEOSARCOMA (OS).

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With the more successful treatment of OS initiated in the early 1970s, it was inevitable that some survivors would develop SMN. Eight patients (pts) among 330 have developed SMNs, including two with colorectal carcinoma and one each with gastric carcinoma, secondary OS, chondrosarcoma, glioblastoma multiforme, melanoma and malignant fibrous histiocytoma (MFH). Seven of eight pts were males. Median age of primary OS was 14.6 years (range 12.6 - 18.4 yrs). Median age of SMN was 20.9 years (range 13.9 -34.7 yrs), and median interval between OS and SMN was 5.6 years (range 0.5 - 17.8 yr). Two pts with lung metastasis at diagnosis were rendered disease-free by pulmonary metastasectomy and amputation. Adjuvant chemotherapy given to each of these pts included: VCR/Cyclo (1); BCD-/HDMTX/Doxo/DDP (2); Ifos/HDMTX/Doxo/DDP (1); HDMTX/Doxo/Cyclo (3) and Ifos/Carbo/HDMTX/Doxo(1). Only the pt with secondary melanoma had not completed adjuvant chemotherapy prior to developing his SMN. Radiation therapy was not used to treat the primary malignancy. The cumulative incidence of SMN at 10 years for OS pts with and without metastases at diagnosis were 9% (± 7%) and 2% (± 1%) respectively (p=0.099). Five pts died, four because of their second neoplasm, and one from recurrence of both colon carcinoma and OS. Three pts survive (one each with secondary rectal cancer, melanoma and MFH). Two pts in this series had histories consistent with germ line p53 abnormality, including family members with breast, colon, osteosarcoma and rhabdomyosarcoma, and one of these had proven p53 germ line mutation. Although the prognosis for high grade SMNs is poor, identification of OS pts with family cancer syndromes may lead to earlier diagnosis and more successful treatment.(Supported by USPHS Grants CA-23099, CA-21765 and by American Lebanese Syrian Associated Charities [ALSAC]).

O-26

BONE AND SOFT TISSUE SECOND TUMOR AFTER RETINOBLASTOMA. THE EXPERIENCE OF INSTITUT CURIE

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427 patients (pts) with retinoblastoma (Rb) were treated at Institut Curie between 1971 and 1988. 20 of them (4.7 %) developped a second tumor.

Patients and treatment of retinoblastoma : There were 13 boys and 7 girls aged from 4 to 36 months (mo) at diagnosis (med = 11). 19/20 were bilateral, 5/20 familial cases. After first line therapy 9 local relapses were encountered. In the whole 1 - Enucleation was necessary for 21 eyes in 18 pts (including 5 relapses). 2 - External beam radiotherapy was given to 19 pts, bilateral in 11, unilateral in 8, at a median dose of 45 Gy (45-52 Gy). 3 - 9 pts received chemotherapy (CT) : vincristin and cyclo or ifosfamide (I) (9) actinomycin D (4) adriamycin (A) VP16 Cis platine (P) (1). Growth hormon was delivered to 3 pts.

Bone and soft tissue tumors : they occured 51 to 216 mo (med = 129) after the diagnosis of Rb and were located within (16) or distant from (3) the irradiation field. Histological diagnosis was osteogenic (11) or soft tissue (9) sarcoma. The tumor was localised in 18 pts, with regional lymph nodes or with bone and subcutaneous metastases in one each. In 16 evaluable pts complete excision of the tumor was made in 9, at diagnosis (3) or on a post CT residue with immediate bone reconstruction (6). CT (I.A.P) was given to all pts, with HD MTX to osteogenic and VP16 to soft tissue sarcomas. 2 pts received an intensive therapy.

Therapeutic results : 3 pts are still on treatment and one suffered a toxic death at d2. In 16 evaluable pts there were 8 CR, 1 minor response, 1 SD, 6 PD. 10 pts died of disease 4 to 28 mo (med = 9) from diagnosis. 6 are alive, five in first CR at 25, 34, 60, 86 and 142 mo, one in third CR at 12 mo from the last local relapse.

In conclusion : appropriate CT followed by complete surgery is worthwhile in second tumor following Rb since long term survival can be expected in a third of the pts.

O-27

THE SIOP 93-01 WILMS' TUMOR (WT) TRIAL AND STUDY PROTOCOL, A EUROPEAN UNION CONCERTED ACTION.

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The SIOP 93-01 trial and study was started in July 1993. Treatment strategy is based on the previous trial results of the SIOP. **Preop. chemotherapy** is given to all newly diagnosed WT patients older than 6 months : 4 wks Actinomycin D and Vincristine **(AV) if localized** and 6 wks AV + Anthracyclin[1] **(AVE) if metastatic. Postop. treatment** is **adapted to different risk groups** taking into account the SIOP histological classification[2], stage and response to preop. treatment in stage IV. There is no postop. therapy in low risk patients stage I. In **stage I intermediate risk and anaplasia,** after 4 wks AV postop. therapy, **2 more maintenance AV** courses are **randomized** versus **no more therapy.** In **intermediate risk** WT, patients with **Stage IIN0, IIN+, III and IV** in CR after preop. treatment, receive the **AVE** protocol. **abdominal RT is limited to Stage IIN+ and III.** In **anaplastic stage over I and CCS** all stages or in stage IV **"bad responder"** a combination of Anthracyclin[1], Ifosfamide, VP16 and Carboplatin is given. So far, **524** Pts are registered from **112** centres and **15** countries. **324** pts are assigned to the AV preop treatment and **43** stage IV pts to the AVE one, **157** are registered as study pts. Over **150** pts eligible for the stage I trial, **92** are randomized and **58** excluded : *the main reasons for non* inclusion are parent refusal : **34** and doctor preference : **21**. In stage I and in high risk pts, the results are too preliminary for conclusion. In the other groups SIOP 9 results are being confirmed by 5 yr survival and 5 yr event free survival rates in stage I low risk of both 100%, stage IIN0 intermediate risk 85% and 81%, stage IIN+,III 89% and 77%.

(1) Adriamycin if GPOH, Epirubicin if other centers, (2) MPO 26 : 145-146 (1996)

O-28

A COMPARISON BETWEEN SINGLE DOSE AND DIVIDED DOSE ADMINISTRATION OF DACTINOMYCIN AND DOXORUBICIN. A REPORT FROM THE NATIONAL WILMS TUMOR STUDY GROUP.

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Between August 16, 1986 and September 1, 1994, 1688 previously untreated children less than 16 years of age with stages I-II/favorable histology (FH) or stage I/focal anaplastic Wilms tumor (WT) ("low-risk")(LR) or stages III-IV/FH WT or stages I-IV/clear cell sarcoma of the kidney ("high-risk")(HR) were randomized to treatment which included vincristine and either divided dose ("standard")(STD) courses (5 days) or single dose ("pulse-intensive")(PI) treatment with dactinomycin. HR patients also received either divided dose (STD) courses (3 days) or single dose (PI) treatment with doxorubicin. The two-year relapse-free survival (RFS) percentages for LR patients were 90.8 (± 1.3) for 542 treated with PI and 91.2 (± 1.3) for 554 treated with STD chemotherapy (p = 0.89). The two-year RFS percentages for HR patients were 86.7 (± 2.1) for 301 treated with PI and 89.1 (± 2.0) for 291 treated with STD chemotherapy (p = 0.73). We conclude that patients treated with PI combination chemotherapy for Wilms tumor have equivalent two-year relapse-free survival to those treated with STD regimens. PI drug administration is recommended as the new standard based on demonstrated efficacy, greater administered dose-intensity and less severe hematological toxicity (Am J Pediatr Hematol Oncol 1994; 16:207-212), and the requirement for fewer physician and hospital encounters, resulting in lower costs (Monogr Natl Cancer Inst 1995;19:21-25).

O-29

LONG-TERM RESULTS OF THE FIRST ITALIAN COOPERATIVE WILMS' TUMOR STUDY

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This prospective, non-randomized study was undertaken with the following aims: to apply in a multicenter setting, in Italy, a standard *therapeutic protocol for the treatment of Wilms' tumor and to assess the* feasibility and the therapeutic impact of the protocol. Between January 1980 and October 1987, 209 patients > 6 mos and < 16 yrs of age, with a first diagnosis of stage I-IV nephroblastoma were registered by 14 Centers. 185/209 were considered eligible to the study and subsequently evaluated (75 males; median age: 39 mos). According to *NWTS criteria,* stage classification was as follows: stage I 62 pts, II 52, III 60, IV 11. Thirty of 185 (16%) had an unfavorable histology (UH) tumor. Patients who presented with pulmonary metastases (stage IV) or unresectable tumors received a short course of pre-operative chemotherapy (CT) with VCR *1.5* mg/m^2 + *ActD 15* mcgr/Kg x 5. Adjuvant *CT* was given to *all* pts and consisted of VCR, ActD ± ADR 50 mg/m^2. Treatment schedule and duration depended upon stage and histology. Age and histology-adjusted local/abdominal±lung radiation therapy was given to stages II-IV. Long-term survival (S) and progression-free survival (PFS) were evaluated (median follow-up 9.3 yrs): the 12-yr overall figures were 82% and 77%, respectively. The S and PFS rates by stage were: stage I - 95% and 89%, respectively; stage II - 86% and 78%; stage III - 71% and 66%; stage IV - 64% each. According to histology, S was 88% for FH vs 60% for UH (p <0.001); PFS was 81% vs 60% (p=0.002). Forty children developed recurrent or progressive disease at the following sites: lungs-21 pts, local-9, abdomen-3, multiple sites-3, bones-2, contralateral kidney and uterus-1 each. Twenty-eight pts died for furtherly progressing disease. Two pts died from treatment-related toxicity (hepatopathy-one, sepsis-one) while they were in CR. These results are superimposable to those of the large international studies. The *following late sequelae* were observed: scoliosis-11 pts, amenorrhoea-6, hepatitis-3, pulmonary-3 (+cardiac in one), pubertal retardation-2. So far, no second malignant neoplasm has been reported in this series.

Supported in part by the National Research Council ACRO grants.

O-30

IS CONTRALATERAL EXPLORATION IN WILMS' TUMOUR NECESSARY? - RESULT FROM UKCCSG BILATERAL WILMS' TUMOUR STUDY

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Aim: Surgical management of unilateral Wilms' tumour includes routine exploration of the contralateral kidney. Excellent advances in radioimaging, especially CT scan has improved preoperative detection of tumours in kidneys. In this context, the usefulness of routine contralateral exploration in diagnosis of bilateral lesions is analysed in this paper.

Materials and Methods: Seventy children with bilateral Wilms' tumour (BWT), 16 between 1980-1985 in Wilms' Tumour Trial I (WT1), 23 between 1986-1991 in WT2 and 31 between 1991-1995, were registered with UKCCSG. Sixty-nine of 70 were synchoronous tumours. Ultrasound (US) was done in all 69, CT scan in 55 mostly in WT2 and WT3, MRI in 4 patients in WT3 and IVP in 16 patients in the early part of the series. The follow-up period was 1 to 15 years.

Results: All patients in WT3 were diagnosed preoperatively: three patients in WT1 and WT2 each, were diagnosed at surgery. All six patients who were diagnosed at surgery to have contralateral lesions are alive; the lesions were quite small requiring only biopsy or enucleation. CT scan was accurate in picking up the lesion in 54 of 55 cases with sensitivity of 98% and 100% specificity. In the only case where CT failed to pick up the lesion, it was 4 mm diameter and only biopsy was done with no change in management. The uniform excellent preoperative diagnosis especially in recent years was irrespective of centres analysed reflecting advances in radioimaging.

Conclusion: Routine exploration of contralateral kidney in unilateral Wilms' tumour can be avoided, provided a preoperative CT scan has ruled out a lesion. Even when the lesions are picked up at surgery, no change in management was noted.

O-31

BILATERAL SYNCHRONOUS WILMS' TUMOR (WT) : IS IT A GOOD MODEL OF CONSERVATIVE SURGERY FOR UNILATERAL WT ?

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Total nephrectomy is recommended for treatment of unilateral WT (UWT) in SIOP studies, while for bilateral WT (BWT) conservative surgery (CS) is mandatory. Sparing of at least 2/3 of nephronic mass is a condition to preserve renal function. In order to help CS, SIOP protocol recommends preoperative chemotherapy (CT) using Vincristin and Actinomycin D until maximal regression of the tumors. The aim of this paper is to study if results of CS in non metastatic BWT should be used to advocate or not CS in non metastatic UWT.

Files of children registered in SIOP 6 and SIOP 9 studies in 2 centers over a 12 years period have been reviewed. One case requiring binephrectomy was excluded and 21 cases of synchronous BWT initially planned for CS were included. Following data have been collected: preoperative therapy, surgery, higher local stage, incidence of relapses, crude and disease-free survival and renal function at follow-up.

Children were brought to surgery after 2 to 5 CT courses (mean = 4) with a good response in 37/42 kidneys. Surgery consisted in total nephrectomy for 9/42 kidneys, partial nephrectomy, and/or single or multiple wedge resections for 32/42 kidneys and 1 biopsy (nephroblastomatosis nodule). Higher local stage was I, II or III in 32, 7 and 2 children respectively. Two local and 1 metastatic relapses occured in 2 children, who died. One- and 10-years actuarial crude survival are 100% and 90% and disease-free survival 95% and 90% respectively. With a mean follow-up of 6.6 years, 17 of 19 surviving children have an optimal renal function, 1 has mild renal insufficiency and renal transplantation was required in the last child.

These results are comparable to those of non metastatic UWT treated according the usual protocol, but BWT patients required a mean of 4 versus 2 preoperative CT courses for UWT patients submitted to total nephrectomy. So, results of CS in BWT cannot be directly extrapolated to treatment of UWT.

O-32

BILATERAL WILMS' TUMOUR - RESULTS OF THE UNITED KINGDOM CHILDREN'S CANCER STUDY GROUP (UKCCSG)

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Aim: Bilateral Wilms' Tumour (BWT) constitutes 4-8% of Wilms' tumour, with no standardised treatment. This study was undertaken to provide information which might be useful in formulating management.

Materials and Methods: Seventy-one children with BWT from 17 UKCCSG centres between 1980-1995 were studied. One patient was excluded as the lesion in one kidney was a cystic malformation. Twenty-eight boys and 42 girls between one month and eight years of age were followed up for 1 to 15 years. Details of diagnosis, treatment, prognostic factors and outcome are presented.

Results: Fifty out of 70 children (72%) were alive at the end of study period. Abdominal masses were palpable in 67 and 32 were hypertensive. In all but one, the tumours were synchronous. Other anomalies were noted in 17 boys and 10 girls. Syndromes included WAGR in 5, DRASH in 3, BECKWITH in 2, and PERLMANN in 2.

Fifty-seven underwent initial biopsy followed by chemotherapy and subsequent surgical resection, with the remainder undergoing initial surgical resection.

Two year disease free survival was 70% of 66 children registered between 1980-1993. Twenty-three (82%) males and 27 (64%) females survived the full study period (CHI^2 = 3.5129) and 81% of children who presented at age two years or less survived. There was no difference in survival in those who had initial biopsy followed by chemotherapy and elective surgical resection compared to those who had initial surgical resection. However, 45% of renal mass was preserved in the earlier group compared to 35% in the later group. Sixteen percent survival was noted in children with unfavourable histology (UH) compared to 77% survival with favourable histology ($p<0.01$). The overall survival of children with bilateral stage I disease was 82% ($p < 0.05$).

Conclusions

i. Overall survival in males and children under two years is improved.
ii. Favourable histology and bilateral stage I disease have improved survival.
iii. Initial biopsy followed by chemotherapy and subsequent surgical resection preserves renal mass without altering final outcome.
iv. New approaches in the treatment of UH BWT are necessary.

O-33

PRIMARY NEPHRECTOMY FOR EMERGENCY IN THE SIOP-9 WILMS' TUMOUR PATIENTS

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The SIOP-9 protocol recommends preoperative (pre-op.) chemotherapy in Wilms' tumour (WT) pts over 6 m. of age. However, acute symptoms may lead to emergency surgery with primary nephrectomy. Patients: Between 11/87 and 11/91, 24 of 720 non-metastatic unilateral SIOP-9 pts (3%) developed acute symptoms which led to emergency surgery with primary nephrectomy. Age range - from 10 to 133 months (m=56, x=60). WT side - right in 15 pts and left in 9. WT weight - from 60 to 1420g (m=400, x=447). Results: Acute abdominal complaints were caused by: WT rupture in 13 pts (WT detected prior to - in 10, and at surgery - in 3), painful WT progression mimicking "acute abdomen" in 7, and bowel occlusion in 2 (WT detected prior to surgery). Also 1 pt with unproven WT rupture and 1 with mononucleosis were nephrectomized as emergency cases. Stages: I/2 pts, IINO/5 and III/17 (pre-op. WT rupture - 14; WT rupture during surgery - 2; irradical resection - 1). Histology: standard in 22, unfavourable - 1, favourable - 1. Twenty two pts (92% - stages II & III) received anthracyclines (VCR+ACT+Epirubicine) and 17 (71% - stages III) were also irradiated. Seven complications were registered in 6 pts (25%): intra-operative WT ruptures/2, abscess in the tumour bed/1 (followed by VOD), VOD/3 (all irradiated for the right sided ruptured WT), VCR-related neuropathy/1. Outcome: 2 pts relapsed and died despite the treatment, 22 (92%) are in CR (follow up: 9-79 m. x=48, m=48). Conclusions: Emergency nephrectomies were unfrequent (3%) and were caused mainly by the WT ruptures. Prognosis remains favourable, however, the treatment includes anthracyclines and radiotherapy in most pts. Right-sided stage III pts seem to have an elevated risk of liver toxicity (VOD in 3 of 9).

O-34

COMPLETE NECROSIS INDUCED BY PREOPERATIVE CHEMOTHERAPY IN FORMER STANDARD WILMS TUMOR AS AN INDICATOR OF LOW RISK

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830 pts presenting unilateral Wilms tumor (WT) without anaplastic features registered in the SIOP 9 trial and study received a preoperative chemotherapy. 711 pts free of distant metastases were treated according to the AV protocol using Actinomycin D and Vincristine and 119 metastatic pts were treated according to the AVE protocol where an antracyclin (1) is added. Among them, pathological examination of the tumor after nephrectomy revealed a completely necrotic tumor (CNWT) with no viable tumor cells left in 58 cases, 38 localized WT and 20 stage IV. Only ghost structures of preexisting bi or triphasic WT could be detected on the standard or silver stains. The proportion of CNWT was significantly more important in stage IV pts treated according to a 3-drug and 6 weeks protocol AVE, than in localized WT pts treated according the 2-drug AV one, whatever was the treatment duration 4 weeks or 8 weeks : 17% versus 5% ($p<0.0001$). Comparison failed to show a difference between CNWT and non necrotic WT pts in terms of tumor size at diagnosis (mean volume = 536 versus 461cm3) or weight of the tumor (mean weight =262 versus 327gr). However mean age of the CNWT pts was significantly higher (60 versus 44 mths) ($p<0.002$). Outcome of CNWT pts was very favorable as no event were observed in stage IV pts, 1 pt with a stage IIN+ tumor presented lung metastases who were cured and the only death observed among the 58 CNWT (in a stage I pt) was not related to tumor. 5 yr survival in CNWT pts is 98% versus 89% in non necrotic WT pts. **This observation has conducted the SIOP Wilms tumor pathologist committee to classify CNWT in the low risk group of the SIOP classification of childhood renal tumors(2).**

(1)Adriamycin if GPOH, Epirubicin if other centers, (2) MPO 26 : 145-146 (1996)

O-35

SURGERY ONLY FOR INSS STAGE 2 NEUROBLASTOMA : PROGRESS REPORT FROM THE LOCALIZED NEUROBLASTOMA EUROPEAN STUDY GROUP (LNESG) - SIOP 95.01 STUDY.

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In order to evaluate the safety and efficacy of surgical treatment alone in the management of INSS stage (St) 2 neuroblastoma (NB) without *Nmyc* amplification, an European trial has been initiated. *Patients (pts)* suspected to have a localized NB were registered before surgery. Tumor resectability was evaluated following established radiological guidelines. *Nmyc* copy number as well as disease extension evaluation, using INSS criteria, were mandatory. Prospective evaluation of histopathological subgroup (Shimada), serum LDH level, ploidy and 1p loss of heterozygosity were also recommended. Between January 1995 and 1996, 178 pts have been registered from Austria (15), France (75), Italy (57), Spain (13), Switzerland (7), the Netherlands (1) and the United Kingdom (10). 52% of the pts are boys; primary localization is abdominal in 64% of the cases and median age at diagnosis is 11 months. On January 1996, data on stage and pathological diagnosis are available on 109 pts : 8 had no NB, 7 had ganglioneuroma and 94 had either NB or GNB (37 St 1, 27 St 2, 27 St 3 and 3 St 4 or 4S). Only 24 St 2 pts are eligible in the trial and median follow-up is 4 months. Four relapses have occurred 2 to 6 months after surgery : 3/4 in the primary site and in all cases in metastatic sites (either bone, bone marrow or lymph nodes). 2/4 pts were < 1year of age at diagnosis, 2/4 had unfavorable histology, di or tetraploidy and/or deletion of 1p were present in 3/3 and 2/3, respectively. These preliminary data confirm the importance of careful prospective studies for children with localized NB.

O-36

OPTIMAL TIMING OF PRIMARY TUMOR RESECTION IN HIGH RISK NEUROBLASTOMA

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The optimal extent and timing of resection of the primary tumor in high risk neuroblastoma (INSS stages 3 and 4) remains controversial. Between 1991 and 1995, the Children's Cancer Group recorded 356 such procedures including attempted resection by initial excision (IEX) in 104 patients, by delayed excision (DEL) following chemotherapy in 92 instances, and by a "second look" procedure (2LK) in 160 cases.

	IEX	DEL	2LK	Total
Complete resection (CR)	40	50	67	157
Microscopic residual (MR)	24	21	39	84
Partial resection (PR)	23	12	33	68
Biopsy only (BX)	17	9	18	44
Exploration only	0	0	3	3
Total	104	92	160	356

After statistical analysis, the following significant correlations were noted. Tumors treated by IEX were larger than those treated by either DEL or 2LK ($p<.05$). Both CR ($p<.03$) and CR + MR (no gross residual disease) ($p<.02$) were more frequently achievable by DEL than by IEX. The rate of nephrectomy for at-risk procedures was lower for DEL than for 2LK ($p<.05$) and less likely for CR than for MR ($p<.04$). The risk of other complications was also lower for DEL than for 2LK ($p<.02$) and lower for CR than for all others combined (MR + PR + BX)($p<.03$). Blood loss of 100 cc or more was associated with a higher rate of nephrectomy ($p<.01$), an increased risk of other complications ($p<.0001$), a decreased ability to achieve CR status ($p<.01$), and a greater risk of leaving gross disease (PR + BX)($p<.03$). A two-year survival of approximately 40% appears independent of timing or extent of surgery. Procedures resulting in excessive blood loss increase morbidity. Our preliminary data suggest that delayed resection of the primary tumor in high risk neuroblastoma may be safer than, yet as efficacious as, initial resection. Meaningful comparison to the "second look" procedure requires further analysis.

O-37

IMPROVING SURVIVAL FROM 1978-1995 USING RISK BASED TREATMENT FOR NEUROBLASTOMA: CHILDREN'S CANCER GROUP (CCG).

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Survival for patients with high risk neuroblastoma has remained poor, creating doubt as to whether intensive therapy is really beneficial. To address this issue, we compared survival of patients on CCG protocols from 1978 to 1995, a period during which treatment assignment changed from stratification by stage and age to include biologic factors. CCG-3881 was opened 6/89 for lower risk, and CCG-3891 in 1/91 for high risk. Stage I, II, and IVS had surgery and supportive care, unless symptomatic. Stage IV<1 with single copy N-myc (IVF) and favorable stage III (IIIF= all III<1 yr at diagnosis and III >1 yr with single copy N-myc, favorable Shimada, ferritin <143 ng/ml) received moderate chemotherapy (CCG-3881). Stage IV≥1 yr and stage IV<1 with amplified N-myc (IVU), and unfavorable III (IIIU= ≥1 yr with any unfavorable factor) were treated on CCG-3891 with intensive chemotherapy +/- autologous purged BMT and +/- 13-cis-retinoic acid. Three-yr survival by risk group for 1989-95:

	I	II	IIIF	IIIU	IVF	IVU	IVS
N	138	206	126	56	56	346	80
Survival (%)	99	99	96	71	96	29	89

In comparison, 3-yr survival for all stage III in 1978-1983 was 54% and for 1991-95 was 86% ($p<0.0001$). For stage III >2 yrs at diagnosis, survival was 40% for 1978-83, 36% for 1983-88, and 86% for 1991-1995 ($p=0.0003$). All stage IV < 1 yr 1989-95 had survival of 69%, compared to 51% for earlier patients ($p=0.02$). For stage IV > 1yr, 1978-1988 gave 3-yr survival of 11%, while studies from 1986-95 had survival of 37% ($p<0.0001$). Excellent survival of lower risk disease has been maintained or improved with less therapy. Biological risk assessment has allowed more intensive therapy for high risk groups, resulting in significant improvements in survival for regional and metastatic neuroblastoma.

O-38

PULMONARY NODULES IN CHILDHOOD TUMORS - WHO NEEDS A BIOPSY?

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The lungs are a frequent site of metastases from childhood solid tumors. However malignancy is not the only cause of pulmonary nodules detected on CT especially with helical scanning and there are no clear guidelines as to when to biopsy. We undertook a retrospective review to attempt to establish clinically useful guidelines. We correlated all available CT scans of patients with solid tumors (n=52) who underwent thoracotomies (n=74) over a 6-year period,with the pathology. 333 lung nodules were seen on chest CT, 151 (45%) were removed, 80% were 10mm or less in size. 39% of thoracotomies were for solitary nodules, 61% for multiple. 18 (12%) of nodules were benign, all were 10mm or less in size. Of the 104 nodules 10mm in size or less, however, 86 (83%) were malignant. Of 45 thoracotomies for multiple nodules 4 (10%) showed benign pathology, (despite a history of previous metastases in 2). All had 5 or less nodules, but we have previously seen 2 other cases with multiple (>5) benign nodules, 1 with hamartomas in a Wilms' patient and 1 with chondromas associated with Carney's triad. In 2 patients, both benign and malignant nodules were removed and in 2 additional patients with 2 and 3 nodules respectively on CT, no lesion was found at surgery. 1 osteosarcoma patient had calcificationin a benign nodule. 22 patients had pathologically proven metastases at diagnosis, including 4 of 5 Wilms' tumors, all 4 Hepatoblastomas, all 6 Ewing's sarcomas, 2 of 3 Rhabdomyosarcomas, but only 2 of 16 osteosarcomas. The mean time to metastases in the other 25 patients was 36months (range 0-92). In conclusion, high-grade and chemosensitive tumors tended to present with their metastases at diagnosis. Less chemosensitive and lower-grade, tended to present later, some as late as 92 months. In this group of patients, no CT features including size of nodules, number, site, and the presence or absence of calcification, clearly distinguished benign from malignant. All children with cancer presenting with lung nodules should undergo biopsy if the diagnosis mandates a change in therapy.

O-39

SURGERY ALONE IS EFFECTIVE TREATMENT OF RESECTED IMMATURE TERATOMA (IT) IN CHILDREN: A PEDIATRIC INTERGROUP REPORT (POG9048/CCG8891).

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The potential for malignant recurrence from IT often prompts use of adjuvant chemotherapy following resection of the primary tumor. However, the need for post-operative chemotherapy in children and adolescents with IT has not been proven. To address this issue, 68 evaluable children with resected IT were followed with no further treatment. Central pathology review confirmed histology, graded for immature neural elements (G), and identified microscopic malignant foci in 16/68 (24%); 14 yolk sac (YS) and 2 PNET. Patient ages ranged from <1 month (mo) to 17 years (yrs). Primary sites included ovary (O), testis (T), retroperitoneum (RP), sacrococcygeal (SC), head/neck (HN), or thorax (X). Sites, histologies and ages are shown below:

Site	N	G1(YS)	G2(YS)	G3(YS/PNET)	Median Age
O	40	19(1)	12(3)	9(5/0)	10 Yrs
T	6	4(1)	1	1	8 Mos
RP	7	3(2)	1	3(0/1)	3 Mos
SC	7	1(1)	2	4(0/1)	<1 Mo
HN	6	-	4(1)	2	<1 Mo
X	2	1	1	-	<1 mo, 5 mos

Alpha fetoprotein (aFP) in IT without YS was normal for age in 29, elevated in 20 and unknown in 5; in IT with YS, aFP was normal in 3, elevated in 9, unknown in 2. Estimated 3 yr disease free survival was 96.7% (SE=5.1) at 20 mos median followup. Of the 3 patients who recurred, 1 had aFP of 1042, 1 had foci of YS and 1 had foci of PNET; all were salvaged successfully with chemotherapy. These preliminary data suggest 1) that surgical resection alone will result in cure for most IT patients with (51/52) or without (14/16) malignant elements, regardless of histologic grade; and 2) that the use of post-operative chemotherapy, and its associated side effects, may be avoided in the majority of these patients.

O-40

TERATOMA IN CHILDHOOD AND ADOLESCENCE - A RANDOMIZED COOPERATIVE INTERNATIONAL STUDY FOR CHEMOTHERAPY IN IMMATURE TERATOMA (SIOP TERATOMA 95)

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Mature and immature teratoma are the most problematic and controversial „members" among germ cell tumors. They could be completely benign or potential malignant tumors according to their grade of immaturity. Teratoma are divided into mature and immature grade 1-3 tumors according to Gonzales-Crussi. Besides immaturity, primary tumor site and tumor resection are of prognostic value.

Chemotherapeutic treatment of immature teratoma grade 2 and 3 because of their progression potency have been initiated in the 70ies mainly with adjuvant chemotherapy preferring the VAC-regimen. In the 80ies teratoma were mainly not included in germ cell tumor protocols except if malignant relapses occured.

To optimize treatment in these tumor entities international cooperation was established to create common guidelines for diagnostic and treatment. As basis of this common study results of 277 pts registered in the German MAKEI 83 to 89 protocol between october 83 and december 95 were evaluated prospectively in respect to complete/incomplete resection, grade of immaturity and localisation.

127 pts had sacrococcygeal tumors, 89 children offered ovarian tumors and 61 children had tumors of other locations. 37 of 277 pts developed recurrence, which was malignant in 40%. In these pts in addition to the teratoma mainly yolk sac tumor histology was observed (14/16 pts). The highest risk factor for relapse was incomplete resection. 40% of the so treated pts relapsed. In respect to primary site pts with sacrococcygeal tumors had the highest relapse risk (21%). In accordance to grading mainly pts with teratoma grade 2 and 3 offered recurrence (30%).

To estimate the relapse rate in teratoma a scoring system was employed regarding these 3 risk factors: incomplete tumor resection, primary tumor sites and grade of immaturity. In the cooperative protocol pts then will be randomized for chemotherapy or for no chemotherapy to estimate the influence of this treatment: on occurence of malignant relapse and on relapse risk in general. Adjuvant chemotherapy will consist of 2 courses cisplatin 20 mg/m² day 1-5, VP16 100 mg/m² day 1-3 and ifosfamide 1.500 mg/m² day 1-5.

O-41

THE UKCCSG'S GERM CELL TUMOUR (GCT) STUDIES: CARBOPLATIN, ETOPOSIDE AND BLEOMYCIN ARE AS EFFECTIVE AS AND LESS TOXIC THAN PREVIOUS REGIMENS FOR MALIGNANT EXTRACRANIAL NON-GONADAL TUMOURS.

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Objects - (1) To assess carboplatin, etoposide and bleomycin (JEB) for malignant extracranial non-gonadal GCTs, compared with previous regimens. (2) To evaluate late effects.

Methods - Records of children entered into the 1st (1978-88) and 2nd (1989-95) UKCCSG studies were analysed for clinical presentation, treatment, outcome and long-term toxicities. In the 1st study 3 VAC and 2 platinum-based protocols were used, BEP being given to the largest number (=Day 1 cisplatin 100mg/m², Day 2 bleomycin 15mg/m², Days 1-3 etoposide 120mg/m²). In the 2nd study carboplatin 600mg/m² was substituted for cisplatin (JEB). Actuarial and event-free survival were calculated.

Results - 52/170 and 48/254 children in the 1st and 2nd studies respectively had malignant extracranial non-gonadal GCTs. The sites were, respectively, sacrococcygeal 31 and 28, vagina/uterus/prostate 4 and 3, thorax 7 and 9, other 10 and 8. Five children in each study had yolk sac tumour recurrence of mature teratoma resected in the newborn period.

In the first study (median follow-up 100m) survival was 63% at 5 and 10 years (70% when 5 cases given ineffective low-dose VAC were excluded). In the 2nd study (median f.u. 35m) 5 year survival was 89%. Event-free survival for 18 cases in the 1st study given BEP was 56% (C.I. 33-78%) whereas in 48 children in the 2nd study given JEB it was 64% (C.I. 48-79%). The median number of courses given was 5 both for BEP and JEB.

Late effects in 31 evaluable survivors of the 1st study included renal impairment and deafness, attributed to cisplatin, in 5 and 8 patients respectively. These complications have not been seen following JEB. Among 20 survivors of sacrococcygeal tumours from the 1st study 6 have neuropathic bladder and/or bowel and another has a short leg. Six of these 7 children had neurological problems when the tumour was diagnosed.

Conclusions - JEB is as effective as BEP in children with high-risk extracranial GCTs in children and without renal or ototoxicity but better regimens are needed for these patients. Neurological late effects are significant in survivors of sacrococcygeal tumours.

O-42

SECRETING GERM CELL TUMORS OF THE CENTRAL NERVOUS SYSTEM (CNS GCTS): PRELIMINARY RESULTS OF A COOPERATIVE GERMAN AND ITALIAN STUDY

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Secreting CNS GCTs are formerly poor prognostic tumors. The combination of platinum-based chemotherapy with surgery and radiotherapy improved survival rates in the last years to about 60% (Neuropediat 25: 26-31 (1994)). International cooperation was started in 1993 to initiate a common protocol for diagnostic and treatment. In the background of these efforts German and Italian patients (pts) with secreting CNS GCTs were treated.

Diagnostic, staging and treatment approach: ß-human-chorionic gonadotropin (ß-HCG)>50 IU/l and/or alpha-fetoprotein (AFP)>25 ng/ml in the serum and/or in the CNS-fluid (CSF) were considered as sufficient criteria for diagnosis. Staging procedures included CSF-cytology and MRI (head/spinal). Treatment consisted of 4xPEI: cisplatin 20 mg/m² day 1-5, VP 16 100 mg/m² day 1-3 and ifosfamide 1.5 g/m² day 1-5. Surgery was recommended in case of residue after chemo- before radiotherapy, which consisted of craniospinal irradiation (30 Gy) and tumor boost (20 Gy).

Patient's characteristics: Between January 93 and February 96, 13 (pts), 12 boys and 1 girl aged 10 to 19 years were registered. Diagnosis by markers was done in 4 pts. 4/6 children with significant marker elevation had biopsy showing germinoma. 3 children were primarily resected. CSF-cytology was positive in 3 pts. Raised ß-HCG in CSF was seen in 3, raised AFP in 2 and elevation of both markers in 8 pts. Spinal metastases were detected in 2 pts. All children had increased intracranial pressure, requiring ventricular peritoneal shunt in 7. In 9 pts with diagnosis by markers and/or biopsy the response to chemotherapy was evaluated: In 6/9 children markers were normalized after the 2nd course. Decrease of tumor volume was observed in 7 pts. In 2/7 pts the tumor diminished totally. In one child the tumor size did not change. Despite decrease of markers tumor growth was observed in one pt. In both pts as well as in all children, who received surgery after chemotherapy, mature teratoma was found as residue. Major side effects of treatment were severe infections in 2 pts.

Results: 7 pts have finished their treatment (medium follow-up 16 months) and are in continuous remission. 5 pts are under treatment. One pt died of tumor bleeding.

Conclusions: These preliminary results seem to indicate that the adapted treatment is effective and tolerable. Questions remain on the rationale of extended-field radiation and on its potential long-term side effects. The planned SIOP CNS GCT Study will allow to address more properly these issues.

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PROGNOSTIC FACTORS AND STAGING SYSTEMS IN HEPATOBLASTOMA

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A widely accepted pre-treatment staging system and clearly defined prognostic factors for hepatoblastoma are warranted in order to make results of cooperative trials comparable. We therefore analysed the prospectively collected data of 72 patients treated according to the protocol of the German Pediatric Liver Tumour Study HB89.
54 patients (75%) are disease-free and 18 (25%) died. Distribution and extension of the tumours in the liver, vascular invasion, distant and lymphnode metastases, serum levels of α-fetoprotein, and epithelial differentiation were significant prognostic factors while the tumours' size, number of involved liver segments, and histological subtype were not predictive for outcome. In multivariate analysis with the Cox' regression model and the RECPAM model the tumours' distribution in the liver and serum-α-feto-protein remained independent significant factors.
The conventional post-operative staging was clearly predictive for outcome ($p=0.0009$) while the SIOPEL pre-treatment grouping system ($p=0.0515$) and the Japanese TNM-system ($p=0.0164$), the latter of which is based on the number of involved liver segments, were of limited relevance. In contrast, the TNM-system for adult liver carcinoma, which includes most of the investigated prognostic factors, displayed a highly significant prognostic value ($p<0.0001$) for outcome of hepatoblastoma patients.
We therefore propose the application of this system in all cooperative trials on childhood hepatoblastoma including a G-grading for the tumours' epithelial differentiation and R-categories for post-operative residual disease.

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LIVER SPARING SURGERY FOR HEPATOBLASTOMA

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Surgical resection of hepatoblastomas (HB) is classically based on major hepatectomies removing one or more segments (according to Couinaud's definition) free of disease together with the tumor. Usual chemosensibility of HB makes the need for such "safety margins" often unnecessary. Preoperative chemotherapy resulting in shrinkage of tumor, progress in knowledge and imaging of liver anatomy, and modern surgical techniques allow a more conservative surgery restricted to involved liver segments.

From 1985 to 1995, 13 children aged 6 months to 9 years have been planned for such liver sparing surgery (LSS) for HB after preoperative chemotherapy. The technique of total vascular exclusion was used in all cases. Crude survival, event-free survival and complications have been reviewed.

LSS could be achieved in all 13 children. The number of preserved liver segments ranged from 0.5 (one half of quadrate lobe) to 5, with a mean of 1.8 segments. Crude 2-year actuarial survival was 85% and event-free survival 74%. One child, found retrospectively to have portal vein involvement, died after local recurrence and lung metastases. Another child is alive 10 years after excision of a liver recurrence. The single complication related to LSS was a postoperative bleeding requiring reoperation.

These results suggest that the outcome after LSS is comparable to that reported in other HB studies, and that this choice should be recommended to trained surgeons.

O-45

PROGNOSTIC SIGNIFICANCE OF THE INTERNATIONAL HISTO-PATHOLOGICAL CLASSIFICATION OF CHILDHOOD RHABDOMYO-SARCOMA A REPORT OF THE INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY (SIOP) ON MMT84 STUDY.

Terrier-Lacombe MJ, Marsden HB, Van Unnik AJM, Rodary Ch,Rey A, Brunat M, Otten J, Sommelet D, Voute PA, Flamant F, Oberlin O. on behalf of the SIOP MMT committee. (IGR- 94805 Villejuif, France)

The survival of RMS has dramatically improved over the last years following the introduction of multi modal therapy. The most widely accepted clinical variables associated with prognosis are clinical stage, and anatomic site of the tumor. The concept of favourable and unfavourable histologic subtypes of RMS is well documented but the adverse importance of alveolar histology is more controversial.
We report the results of an analysis performed on the previous SIOP study (MMT 84). From January 1984 to January 1989 pts were recruited from 32 participatring centers of pediatric oncology (24 from France, 2 from Netherlands, 3 from Belgium, 2 from Sweden, 2 from Argentina). Patient eligibility included untreated patients younger than 18 years of age with a diagnosis of RMS confirmed by the pathology review committee. Among the 216 patients, 186 are non metastatic cases. Because of the most important prognostic factor is the metastases, this study concerned only the 186 non metastatic cases. The survival curves were calculated using the Kaplan Meier method. We used the International classification of RMS.

			pts	5 year EFS
I	Good prognosis	Botryoïd - leiomyomatoïd	23	78 %
II	Intermediate	Dense well differentiated	66	67 %
		Dense poorly differentiated	70	46 %
III	Poor prognosis	Classical and solid alveolar	27	33 %

Trend for survival according to histology was significant ($p = 0.005$), even after adjustment on the other prognostic variables.
In this study, inspite of a limited number of cases for analysis, we conclude that alveolar histology has an adverse prognostic value. Furthermore, cellular differentiation within RMS (RMSE) also has prognostic importance. Dense well differentiated RMSE have a survival approaching botryoïd RMSE, whilst dense poorly differentiated RMSE has a survival close to that of alveolar RMS. The implications of these findings in relation to solid alveolar variant of RMS and to the recently proposed International classification will be discussed.

O-46

OUTCOME OF CHILDREN WITH PRESENCE OF DIFFERENTIATED RHABDOMYOBLASTS AT THE END OF THERAPY (TX) FOR RHABDOMYOSARCOMA (RMS)

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Presence of differentiated rhabdomyoblast in biopsy material obtainined at end of TX for RMS has created a dilemma whether or not it should beconsidered as evidence of active tumor. For the purpose of defining the potential malignant nature of these differentiated rhabdomyoblasts six children with pelvic RMS whose biopsy material at end of the precribed TX demonstrated this finding as the only evidenceof tumor were closely monitored for disease activity. They were evaluated with serial endoscopic examination and biopsies of tumor primary site and results were related to final outcome. All six patients were clinical group III at diagnosis (DX) and the tumor histology of embryonal type. In four patients the tumor originated from the bladder/prostate(B/P) and in two from the vagina (VAG). The TX received and its date of completetion, the dates of endoscopy and biopsy and the present status of the six patients are as follows:

Pts.	Dx	Primary Site	Chemotherapy	XRT	TX Completed	End of and off TX Biopsies	Status
1	1/74	B/P	VACx21/Adr.	Yes	7/77	3/75,3/76,8/78,7/80	NED
2	1/75	B/P	VACx26	Yes	3/78	1/77,7/77,9/77,1/78	NED
3	10/88	VAG	Reg 37 IRS	Yes	2/91	4/89,8/89,4/91	NED
4	10/90	B/P	IVA/CARBO-Adr	Yes	9/93	12/92,3/92,6/92,11/895	NED
5	12/90	B/P	Reg C IRS IV Pil.	Yes	6/92	1/92,3/92,6/92,992,12/92 4/93,9/94	NED
6	11/92	VAG	Reg 41 IRS V	No	11/93	3/93,11/93	NED

Five patients had their TX extended beyond its original prescribed length because of the finding of differentiated rhabdomyoblasts at the end of TX. The end of and off therapy biopsies material were characterized by presence of residual rhabdomyoblasts with features of differentiation including large single or multiple nuclei, large amount of pinkish granular cytoplasm, some with cross striation, and absence of mitotic activity. Immunohistochemical staining for muscle specific Actin, Desmin and Myoglobin did not help in defining the nature of these cells. The number of differentiated rhabdomyoblast appear to decrease with each subsequent biopsy. All six patients are alive and free of disease off therapy from 2 to 18 years. We conclude that 1.) presence of mature rhabdomyoblasts at the end of therapy does not imply persistence of the malignant process and 2.) is not an indication for conituation of therapy. This experience shoudl be prospectivel confirmed in a large number of patients.

O-47

MUTATION PATTERN OF THE RB1, P53, MDM2 AND SAS GENES IN OSTEOSARCOMA (OS).

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Antioncogene deletion and protooncogene activation are the central events of tumorigenesis. Different patterns leading to clinically identical results are of principle biologic interest. We examined the cell cycle regulating tumor supressor genes RB1 und p53 and the oncogenes MDM2 (Murine Double Minute 2) and SAS (Sarcoma Amplified Sequence). MDM2 and SAS are located at 12q13, a chromosomal region frequently amplified in human sarcomas. Overexpression of the MDM2 oncogene product is assumed to inhibit p53 function and was described in relapsing OS. SAS is thought to participate in signal transduction during growth control. We investigated the DNA of 20 OS (17 high and 3 low-grade OS) for gross RB1 and p53 alterations and increased copy numbers of the oncogenes by Southern blot analysis. P 53 point mutations in exons 5,6,7, and 8 were identified by Temperature Gradient Gel Electrophoresis (TGGE) prior to direct sequencing. In 13 cases immunhistological analysis (IHC) for abnormal p53 protein were also performed. We found in 8/20 OS RB1 mutations and in 13/20 OS p53 alterations (including 3 low grade OS), three of which were due to MDM2 amplification. One of these 3 OS was examined for p53 protein expression and showed a positive IHC result indicating that MDM2 amplification may cause p53 protein stabilization. SAS amplification was found in only one OS suggesting a minor importance during OS genesis.

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O-48

IN VITRO TREATMENTS AGAINST IGF-I RECEPTOR INHIBITS GROWTH OF EWING'S SARCOMA CELLS

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The need for innovative therapeutic strategies for patients with Ewing's sarcoma prompted us to investigate the existence of autocrine and/or paracrine circuits to be used as possible targets. The expression of different growth factors (IGF-I, IGF-II, EGF, NGF, TGFα, TGFβ1, PDGF, and FGF) was analyzed by RT-PCR, and the expression of their receptors was evaluated by immunofluorescence in 7 cell lines and 10 frozen samples of Ewing's sarcoma. High levels of TGFβ1 were found in all of the 17 samples. IGF-I, IGF-II, PDGF, FGF, and NGF were also highly expressed in the majority of the samples, whereas both TGFα and EGF showed a more restricted pattern of expression. However, when the presence of their corresponding membrane receptors was analyzed, only the IGF-I receptor-mediated circuit was constantly found in all of the cell lines and tissue samples, suggesting a role for this autocrine loop in the pathogenesis of Ewing's sarcoma. The *in vitro* inhibition of the IGF-I receptor-mediated circuit by suramin or by the specific receptor binding antibody αIR-3 inhibited the growth of Ewing's sarcoma cells. These data indicate that the use of agents capable to inhibit the IGF-I receptor signaling pathway may find application in the treatment of Ewing's sarcoma.

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O-49

ALLELIC LOSS ON CHROMOSOME ARM 1P IN HEPATOBLASTOMA.

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In a recent molecular genetic study, we have found loss of heterozygosity (LOH) on chromosome arm 11p in 33% of hepatoblastomas (HB) (*Cancer Res. 54:5041-44, 1994*). Loss of material of chromosome arm 1p have been reported in single cases. Using a combination of sensitive molecular methods to assess allelic loss on chromosome 1p, we analysed 30 sporadic and two familial HBs enrolled in the German Cooperative Pediatric Liver Tumor Study HB89. PCR-mediated analysis of 10 microsatellites on the short arm of chromosome 1 exhibited loss of heterozygosity in 7/32 (22%) cases with an overlapping region at 1p36.3. Nineteen HB samples were also studied by fluorescence in situ hybridization (FISH) on interphase nuclei with biotinylated and digoxigenin labelled DNA probes D1Z1/D1Z2 located at the centromere and on 1p36.33, respectively. FISH experiments revealed an altered ratio of the two DNA probes indicating loss of genetic material in 8/19 HB samples. In 6 samples, only minor fractions of HB cells showed alterations in FISH analysis. These subpopulations were missed in our LOH studies. In two samples, LOH was detected by microsatellite analysis but not by FISH. In these cases the altered region may be not covered by D1Z2 or may represent a partial iso-disomy of 1p36.33. Therefore, these results advocate the need for a combination of both methods, FISH and microsatellite analysis. Our data suggest that tumor suppressor genes located in the chromosomal region 1p36.3 are frequently deleted in HB and may contribute to its pathogenesis. The responsible genes remain to be identified.

O-50

IS LATENT MEMBRANE PROTEIN-1 (LMP-1) RESPONSIBLE FOR THE CREATION OF HODGKIN'S AND REED-STERNBERG (HRS) CELLS IN NON-HODGKIN'S LYMPHOMAS (NHL)?

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The aetiology and origin of the HRS cells is still an enigma. As the part of our HD study we found three high grade NHL with typical HRS cells in children. Despite this finding, the background histology in all three cases was not compatible with a diagnosis of HD. The first patient was a 10 years old girl with history of idiopathic thrombocytopenia (ITP) and splenomegaly 3 years before she developed NHL with HRS cell. The second child was a 7 years old boy with 1/2 year history of uncontrollable ITP treated with splenectomy before he developed NHL of cervical lymph nodes. The third patient was a 13 years old boy with 4 months history of swelling in neck after fever.

The histology of all cases was carefully reviewed in conjunction with immunotyping studies using a panel of monoclonal and polyclonal antibodies. The large cells were positive for CD20, CD74, CD75, CD30 but negative for EMA, CD3 and CD15.

We investigated for presence of EBV using *in situ* hybridisation for two small untranslated RNA species termed the EBV-encoded RNAs (EBERs) and we applied immunohistochemical staining for EBV latent membrane protein 1(LMP-1) using a cocktail of four monoclonal antibodies CS1-4. EBERs transcripts were detected in HRS cells and lymphoblasts. However, only HRS cells were found to be LMP-1 positive.

Thus since LMP-1 is consistently expressed in HRS cells and is essential for B-lymphocyte growth transformation, we strongly believe that LMP-1 is the key effector in transformation of small lymphocytes and may be responsible for the creation of HRS

O-51

ALLELIC LOSS OF CHROMOSOME 1p36 REGION IS A RARE EVENT IN EWING'S TUMORS

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Purpose: The rearrangement of chromosome 1 is the most frequent cytogenetic abnormality of solid pediatric tumors present in 50% of neuroblastomas of stage IV. Since there are cytogenetic reports, which have shown the occurance of 1p deletions in a subset of ETs, we examined 24 ETs for the occurrance of chromosome 1p rearrangements using PCR based microsatellite analysis.

Material and Methods: Tumor specimen with proven t(11;22) or t(21;22) translocation and constitutional white blood cells from 24 ET patients were analyzed at 6 microsatellite loci dispersed along the short arm of chromosome 1 to which putative neuroblastoma tumor suppressor genes Nb-R1 and Nb-R2 have been linked. Microsatellite regions D1S243, D1S214, D1S234, D1S255, D1S200, D1S203 were amplified by PCR using CY5 fluorescently labelled primers. PCR products were separated electrophoretically with on-line detection and subsequent fragment analysis and data collection on an automated DNA sequencer (A.L.F. Express, Pharmacia). Aneuploidy of chromosome 1 was examined by interphase cytogenetic analysis using chromosome 1 alpha-satellite probes.

Results: All cases were found to be informative for at least one locus in both the Nb-R1 and Nb-R2 region. Reduction to homozygosity of 1p36 was detected in 2 out of 24 pts. In 1 case allelic loss was found in all loci of Nb-R1 and Nb-R2 region, indicating that this tumor has undergone deletion of most of the short arm of chromosome 1. In the other case LOH was observed at the telomeric loci D1S243 and D1S255 of the Nb-R1 region, whereas heterozygosity was found in all loci of Nb-R2. In all cases, no numeric chromosome 1 alterations were detected neither through differences in the relative amount of the peak areas of the PCR products obtained by amplification of the different alleles, nor by FISH using a chromosome 1 alpha-satellite probe.

Conclusions: In contrast to recent publication these results suggest that LOH of 1p36 region with the putative neuroblastoma tumor suppressor genes Nb-R1 and Nb-R2 represents a rare event in the development and progression of ETs.

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O-52

EXPRESSION OF THE EWS-FLI1 FUSION GENE PRODUCT IN NEUROBLASTOMA.

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The t(11.22)(q12.24) results in expression of a chimeric RNA product, EWS-FLI1. This transcript is increasingly used as a definitive characteristic of the Ewing's and peripheral primitive neuroectoderm family of tumours (pPNET). RNA from twelve catecholamine secreting neuroblastomas and six pPNETs was reverse transcribed to produce cDNA. Using the polymerase chain reaction, this cDNA was amplified for the EWS-FLI1 fusion product. Extraction of RNA, subsequent reverse transcriptase polymerase chain reaction (RT-PCR) and analysis of products was performed in designated lamina flowhoods and rooms. The identity of amplified products was confirmed by liquid hybridisation and sequence analysis. All pPNETs analysed were positive for EWS-FLI1 mRNA, this positivity correlated with conventional cytogenetic analysis. RNA from 2/12 neuroblastomas expressed EWS-FLI1 fusion transcripts. These two neuroblastomas were localised in the adrenals of a 5.5 and a 3 year old patient. The presence of EWS-FLI1 mRNA in neuroblastomas contradicts the suggestion EWS-FLI1 transcripts are pathogonomic of Ewings and pPNET. The clinical significance of these transcripts in neuroblastoma is not known, though both patients described in this report with these transcripts had aggressive malignant disease of which they subsequently died. Molecular characterisation of tumours of neuroectoderm origin may help characterise tumours into more significant clinical groups.

O-53

BINDING OF A HU FAMILY PROTEIN TO N-*myc* and c-*fos* mRNA CORRELATES WITH ENHANCED TUMORIGENICITY IN HUMAN NEUROBLASTOMA

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NBL-W, an N-*myc*-amplified human neuroblastoma (NB) cell line, contains two biochemically and phenotypically distinct subclones (N and S). N cells express 5-fold more N-*myc* mRNA and protein than S cells, and only N cells are tumorigenic *in vivo*. We have determined that N-*myc* mRNA turnover plays a major role in the regulation of steady-state N-*myc* expression in NBL-W. The half-life of N-*myc* mRNA is ~30 minutes in N cells compared to ~6 minutes in S cells. Half-life studies also show that c-*fos* mRNA is more stable in N cells than S cells. Using RNA gel shift and cross-linking assays we have identified a 40-kD protein in N cells that specifically interacts with 2 AU-rich elements within the N-*myc* 3' untranslated region (UTR), and at least 1 AU-rich element within the c-*fos* 3'UTR. This protein is not detected in extracts from S cells. Screening of 9 additional human NB cells lines and 19 primary NB tumors revealed a strong correlation between 40-kD complex formation and tumorigenicity. Of the 5 patients whose tumors were positive; 4 have died from disease. Thirteen of the 14 patients whose tumors were negative remain disease-free for more than 33 months from diagnosis. Immunoprecipitation experiments performed with a series of antibodies that react with known RNA-binding proteins demonstrated that only anti-Hu antibodies react with the 40-kD RNA-protein complex. The Hu family of neuron-specific RNA-binding proteins (HuD, HuC/pLE21, Hel-N1) are believed to be involved in the development and maintenance of neurons. We hypothesize that Hu proteins may also regulate N-*myc* and c-*fos* expression by controlling turnover of the message, and may thereby play a role in determining the biologic behavior of NB.

O-54

LOCALIZED NEUROBLASTOMA: STRONG PROGNOSTIC VALUE OF THE LOSS OF CHROMOSOME 1p (LOH 1P).

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on the behalf of the *Société Française d' Oncologie Pédiatrique*.

Between March 1990 and December 1995, 316 consecutive children with localized NB have been registered in the french NBL 90 study. In addition to the assessment of new chemotherapy regimen in unresectable NBs, we evaluated the prognostic significance of N-Myc amplification (NMA) and loss of the short arm of chromosome 1 (LOH 1p).
Results: NMA was found in 22/225 children (10%) and associated with unfavorable clinical features such as age>1 year, large and unresectable tumors, lymph node (LN) involvement, and poor outcome (Event-Free Survival, EFS=33% vs 89% in children without NMA, $p=8.10^{-8}$). LOH 1p was observed in 12/101 patients (12%), of whom some had favorable prognostic factors such as infants (n=7), INSS stage 1 or 2 (n=5), absence of LN invasion (n=3) and no NMA (n=6). Overall Survival (OS) and EFS were respectively 67+/-22% and 25% (median follow up: 36 months) for children with LOH 1p, compared to 96+/-4% and 92% for the ones without (log rank=8.10^{-8}). Among the 8 patients with NMA explored for LOH 1p, 2 had no deletion. Out of the 6 children with NMA and LOH 1p, 5 relapsed, of whom 4 ultimately died of the disease. Of the 6 with LOH 1p and no NMA, recurrence occured in 4 (2 local, 2 metastatic), all alive in 2nd remission after salvage therapy (9-18 months after the relapse). In multivariate analysis, LOH 1p was the unique significant prognostic factor to influence adversely the outcome.
Conclusion: LOH 1p appears more discriminant than NMA to predict the risk of relapse in children with localized NB. However, its analysis was performed in only 30% of patients and its final impact on survival should be evaluated in larger and prospective studies. It should further help to tailor subsequent treatment.
This work was partly supported by the *Association pour la Recherche contre le Cancer*.

O-55

p16 ALTERATIONS AND DELETION MAPPING OF CHROMOSOME 9 IN NEUROPBLASTOMA

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We previously reported high incidence of loss of heterozygosity (LOH) on chromosomes 2q, 9p, and 18q in neuroblastoma (NB) and patients with 9p LOH in the tumors showed statistically significant association with advanced stage of the disease and poor prognosis independently from N-myc amplification. To determine more clearly the role of chromosome 9 loss in NB, we performed deletion mapping for chromosome 9 in 80 cases of NB using 11 polymorphic microsatellite markers and one polymorphic DNA marker. There were two commonly deleted regions mapped to 9p21-22 between the D9S171 and IFNA marker and 9q34-qter between the D9S176 and telomere. Because one of the commonly deleted region includes the p15 and p16 genes, we examined the genomic status of the p15 and p16 in 80 fresh tumors and 21 cell lines of NB. We detected only one alteration of the p16 gene In addition, although patients with 9p21-22 (between D9S171 and IFNA) LOH in the tumors showed statistically significant association with poor prognosis (P=0.041), 9q34-qter (one of the commonly deleted regions, between D9S158 and telomere) LOH was not associated with the prognosis. These results indicated that the p16 gene is one of the candidate tumor suppressor genes and may contribute to the progression of NB. To determine the relationship between the p16 gene and the genesis and/or progression of NB, more detailed analyses will be required.

O-56

A PROPOSED INTERNATIONAL NEUROBLASTOMA PATHOLOGY CLASSIFICATION (INPC)

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A collaborate international work to establish common criteria has so far resulted in an International Neuroblastoma Staging System (INSS) and a set of International Neuroblastoma Response Criteria (INRC). In the continued work for a comparable International Neuroblastoma Risk Grouping (INRG), six pathologists have worked to make an International Neuroblastoma Pathology Classification.
The basic flavor of Shimada's age-linked classification is maintained, ie focus on Schwannian stroma, ganglion cell differentiation and Mitosis Karyorrhexis Index. The new classification is based on the following concepts:
1) Neuroblastic tumors are derived from the neural crest and embryologically related to the sympathetic nervous system and adrenal gland.
2) The Schwannian stromal cells are non-neoplastic and proliferation of these (stroma rich tumours) indicate favorable prognostic features.
3) The neoplastic cells are neuroblastic and can differentiate towards ganglionic cell (enlarged, vesicular, eccentric nucleus with prominent nucleolus, eosinophilic/amphophilic cytoplasm, and diameter of cell ≥ 2x nucleus).
4) The Mitosis Karyorrhexis Index give biological information.
5) Tumor cell nuclear morphology give some biological information.
6) Composite tumours (ie with stroma poor nodules) are grouped separately.
The classification is purely morphological with a basic diagnosis of neuroblastoma, ganglioneuroblastoma or ganglioneuroma. Guidelines for formulating the diagnosis is included. For prognostic categorization into a favorable or unfavorable group, additional information can be included, ie age, MYCN-status, Ip status etc.

O-57

FIVE-YEAR RESULTS OF THE SSG IX PROTOCOL IN EWING'S SARCOM

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for the Scandinavian Sarcoma Group

Introduction In 1990 a new treatment protocol of Ewing's sarcoma, the SSG IX, was activated by the Scandinavian Sarcoma Group. The protocol features an intensive chemotherapy program of four chemotherapy cycles, each consisting of two courses VAI (vincristine, adriamycin, ifosfamide) alternating with one course PAI (cisplatin, adriamycin, ifosfamide) at three weeks interval. Total treatment time is 35 weeks. Local therapy is given at week 9. Inoperable or non-radically operated patients receive hyper-fractionated radiotherapy 1.5 Gy twice daily between chemotherapy courses to a total dose of 42 to 60 Gy, depending on surgical radicality and tumour localisation.

Patients and methods 67 patients are included (47 male, 20 female, mean age 20 years). The tumour (59 M0 and 8 M1) was located as follows: pelvis (12), rib (8), sacrum (7), femur (7), tibia (7), spine (5), scapula (4), fibula (3), foot (3), humerus (3), mandible (2), ulna (1), radius (2), others (3). Soft tissue extension was seen in 87% of the tumours. As local therapy irradiation was given to 29 patients (43%); 45 patients (67%) were objected to surgery which was amputation in 8 and local excision in 37 patients. The type of operation was wide in 24 patients, marginal in 10 and intralesional in 3. Histologic response has been evaluated in 38 tumours: 7 G1, 9 G2, 5 G3, 17 G4.

Results Only 4 local recurrences have been observed (7%). Eight M0 patients (14%) developed distant metastases. The 5-year DFS is 67% for M0 patients and 61% for the whole group (M0+M1); the overall survival is 71% and 67%, respectively. Patients with a good response to chemotherapy (G3/4) have a better DFS than those with a poor response (G1/2) ($p<0.05$). The toxicity (WHO) is acceptable (gastrointestinal G1-2; haematological G3).

Conclusion The SSG IX protocol gives better local control and survival rates than the SSG IV and the most protocols used by other cooperative groups.

O-58

NON METASTATIC EWING'S SARCOMA OF THE PELVIC - THE SFOP EXPERIENCE

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From 01.84 to 11.93, 60 children with pelvic bone Ewing's sarcoma were treated according to three successive chemotherapy SFOP protocols : EW.84 (15 cases), EW.88 (32 cases), and EW.93 (13 cases). The tumor locations were iliac wing in 33 cases, hip joint in 3 cases, pubic rami in 13 cases and sacrum in 11 cases. Four patients had progressive disease after induction chemotherapy and did not receive local treatment. Local treatment was surgery (s) alone in 18 cases, exclusive radiation therapy (RT) in 30 cases and S + RT in 8 cases. The overall survival and the event free survival of the whole population respectively 52% and 50% at 5 years. Patterns of relapses were analysed in the 56 patients who underwent local treatment : 8 patients relapsed locally, 7 had locally relapse and distant metastases and 11 distant metastases only. Survival according with the type of local treatment is datailed in table 1.

	RT	S	RT + S
Overall survival 5 years	48%	70%	71%
Even free survival 5 years	42%	70%	73%
Local control	63%	83%	87%
Number	30	18	8

In addition, no differences in local control or overall survival were observed between the three groups of patients (EW84, EW88, EW93). The impact of the size of tumors, histologi and clinical responses to chemotherapy, dose and radiation therapy fields will be analysed.

O-59

BONE SARCOMAS OF THE HEAD AND NECK IN CHILDREN: ST. JUDE CHILDREN'S RESEARCH HOSPITAL EXPERIENCE.

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We characterized the clinical findings and outcome of children with bone sarcomas of the head and neck, treated at SJCRH between March 1962 and February 1995. Computer-assisted chart review of 601 patients with bone sarcomas, identified 24 cases with tumors arising in maxilla (11), skull (7), mandible (3), orbit (1), and other (2). Median age at diagnosis of the 10 males and 14 females, was 1.6 yr (range, 0.5-24.9). Fourteen patients had osteosarcoma: 8 were primary and 6 occurred after radiation therapy for retinoblastoma (5) and brain tumor (1). Five children had Ewing's sarcoma, fibrosarcoma (3), and malignant fibrous histiocytoma (MFH; 2). Mass was the most frequent presenting symptom in 58%, followed by pain in 25% of the cases. Diagnosis was made within three months of the onset of symptoms in the majority of patients. Diagnostic imaging revealed a soft tissue mass or density in 67% of the cases with or without sclerosis, osteolysis, calcification, and/or bone destruction. When obtained, bone scan showed abnormal radionucleotide uptake by the tumor. None of the 24 patients had evidence of distant metastasis at diagnosis. Treatment included surgery in 22 patients (14 had incomplete resection, 8 had complete/radical surgery) and chemotherapy in 20. Radiotherapy was given to all 5 patients with Ewing's sarcoma, 3 with osteosarcoma, 1 with MFH and 1 with fibrosarcoma. Eleven patients survive at median of 5.8 yr (range 2-20.8 yr) from diagnosis. Thirteen patients died at a median of 1.5 yr (range 1.5 mo-6.3 yr), 12 of whom had evidence of progressive local disease. Of these 12 patients, 10 had biopsy or incomplete resection. We conclude: 1) bone sarcomas of the head and neck are rare in children, and mostly non-metastatic at diagnosis; 2) incomplete resection is associated with rapid disease progression or local relapse; 3) combination of chemotherapy and radiotherapy is indicated for cases with incomplete surgical resection or Ewing's sarcoma; 4) novel approaches for local control are needed.

O-60

RISK FACTORS FOR AND PROGNOSIS AFTER LOCAL RELAPSE (LR) OF OSTEOSARCOMA (OS). B. Kempf-Bielack, S. Bielack, D. Epler, W. Becker, G. Delling, N. Fuchs, U. Heise, R. Kotz, M. Werner, R. Windhager, W. Winkelmann, K. Winkler. Cooperative Osteosarcoma Study Group COSS. Germany/Austria/Switzerland.

Objective: Definition of risk factors for LR after surgery plus neoadjuvant multiagent chemotherapy for primary, localized high-grade central extremity OS. Determination of prognosis after LR. Methods: The data on local control was reviewed for all fully evaluable patients treated for OS within the prospective multicenter studies COSS-80 to COSS-91 between 1980 and 1992. Patients with LR were evaluated for the presence or absence and timing of metastases as well as prognosis. Results: LR occurred in 24/504 evaluable patients (4.8%). Only 1/24 LR developed later than 3 years after tumor surgery (at 64 months). 23 of 24 LR patients also had evidence of systemic spread at some point of time (7 before, 10 simultaneous, 6 after LR). 21/24 LR patients died of their disease, two were alive with and one without evidence of disease. Type of surgery (limb salvage vs. ablative: 7.6% vs. 2.5%) and histological response to preoperative chemotherapy (poor vs. good: 8.1% vs. 2.5%) were significantly related to the incidence of LR ($p < .01$). When both risk factors were combined (limb salvage in cases of poor response), LR developed in 14.3%. While relative tumor size by itself did not significantly influence the LR rate (more vs. less than 1/3 of involved bone: 7.1% vs. 3.6%, $p = .086$), it influenced the LR risk in poorly responding tumors treated by limb salvage (large 6/17 LR vs. small 1/35 LR, $p < .01$). Conclusion: In our multicenter trials, the LR risk in OS was correlated to both type of surgery and tumor-response to primary chemotherapy, with tumor size asserting an additional influence in high-risk situations. Supported by: Bundesministerium für Forschung & Technologie and Deutsche Krebsgesellschaft.

O-61

TREATMENT OF NEWLY DIAGNOSED HIGH GRADE OSTEOSARCOMA (OS) WITH IFOSFAMIDE (IFOS), ADRIAMYCIN (ADR) AND CISPLATIN (CDP) WITHOUT HIGH DOSE METHOTREXATE.

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74 patients (pts) with high grade OS were treated with preoperative chemotherapy regimen of IFOS (1800 mg/m^2/day x 5 with mesna), ADR 25 mg/m^2/d x 3, continuos infusion), and CDP (120 mg/m^2/d). IFOS/ADR was administered on weeks 0, 6, 12 and CDP/ADR on weeks 3 and 9. Surgery was performed on week 15. Postoperatively, etoposide (VP16) was substituted for ADR and all patients were treated with alternating courses q 3 weeks of CDP/VP_{16} (150 mg/m^2/d x 3) and IFOS/VP_{16} (100 mg/m^2/d x 5) for a total of 7 courses. 35 males and 39 females (median age: 12 years; range 3-24 years) with localized OS were entered onto this trial. 28% had osteoblastic morphology, 74% presented in the distal femur or proximal tibia. 62% of pts had limb sparing resections. Histologic response has been assessed in 58 pts: 16 (28%) had 100% necrosis (NEC); 20 (34%) had 95-99% NEC; 4 (7%) had 90-94% NEC and 18 (31%) had <90% NEC. The median follow-up is 2 years (3-45 months). The actuarial progression-free survival is 70% and overall survival is 85% at 3 years. Four non-tumor deaths have been reported: 2 sepsis, 1 acute leukemia, 1 cardiotoxicity. We conclude that IFOS and CDP in combination with ADR for treatment of newly diagnosed localized pts with OS is reasonable and worth further randomized comparison to high dose methotrexate-containing regimens.

O-62

CURATIVE THERAPY FOR OSTEOSARCOMA WITHOUT CISPLATIN - PRELIMINARY EXPERIENCE WITH SJCRH OS-91

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All contemporary therapies for osteosarcoma use multi-agent chemotherapy, typically including cisplatin. In 1991, we began a prospective trial for patients with previously untreated osteosarcoma to evaluate the activity of ifosfamide (IFOS) and carboplatin (CARBO) presurgery ("window") chemotherapy, and estimate the progression-free survival using adjuvant chemotherapy without cisplatin. Prior to surgery, patients receive 3 cycles of IFOS (2.65 g/m^2/d x 3) and CARBO (560 mg/m^2 day 1). Clinical and radiographic responses to IFOS/CARBO are assessed after 2 and 3 cycles (week 6 and 9). Post-surgical chemotherapy comprises hi-dose methotrexate (12 g/m^2 x 9 doses), doxorubicin (75 mg/m^2 x 5 doses, delivered as 72-h continuous i.v. infusions), and two additional cycles of IFOS/CARBO. Patients developing early tumor progression undergo ablative surgery, with substitution of cisplatin for IFOS/CARBO. From Aug. 1991 to Dec. 1995, 38 patients (17 males:21 females) with resectable, non-metastatic osteosarcoma (32), malignant fibrous histiocytoma (2), chondrosarcoma (2), and multipotential tumor of bone (2) were treated. The median age was 14 years, 10 months (range - 9 y, 1 mo to 25 y, 9 mo). Primary sites included: femur (21), tibia (8), humerus (3), fibula (2), other (4). Fifteen of 33 resected tumors evaluated to date show greater than 90% tumor necrosis. Only two patients had tumor progression during the presurgical window therapy and subsequently received cisplatin; one of this group subsequently relapsed. The projected 2-year progression-free survival is 72% (s.d. 11%). This preliminary analysis demonstrates that excellent cure rates, comparable to those achieved with cisplatin based regimens, are possible using carboplatin and ifosfamide-based multiagent chemotherapy. Further investigation of carboplatin substitution for cisplatin is warranted.

O-63

NEOADJUVANT CHEMOTHERAPY (CT) FOR OSTEOSARCOMA OF THE EXTREMITIES (OE) IN PATIENTS AGED 15 OR YOUNGER. RESULTS OF THE 2ND ITALIAN NEOADJUVANT STUDY (OS/IOR2).

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Between October 1986 and December 1989, 84 patients aged 3-15 years with localized Oe were treated according to the OS/IOR2 protocol. Pre-operative CT consisted of Methotrexate (MTX) (8g/m^2/i.v. in 6h), followed 6 days later by Cisplatinum (CDDP) (120 mg/m^2/i.a. in 72h) plus Adriamycin (ADM) (60 mg/m^2 in 8h).The cycle was repeated 3 weeks later and on the 6th week the patients were operated. Three to seven days after surgery all patients received ADM (45 mg/m^2/day for 2 days), then, according to histological response to pre-operative CT, "good responder" patients (tumor necrosis > 90%) had 3 cycles of MTX, CDDP and ADM-used individually per cycle-whereas "poor responder" patients (tumor necrosis < 90%) had 3 cycles of Ifosfamide (IFO) (2g/m^2/day for 5 days), MTX, CDDP plus VP-16 (l00mg/m^2/day for 3 days) and ADM.

Limb salvage was possible in 69 cases (82%), 8 patients (10%) underwent amputation and in 7 (8%) rotation plasty was performed. Surgical margins were radical or wide in 74 patients (88%) and marginal or intralesional in 10 (12%). The rate of good responders was 81%.

Actually 59 patients (70%) are continuous disease-free (CDF) with a minimum follow-up of 6 years, one child (1%) died for cardiotoxicity and 24 patients (29%) relapsed: 17 cases showed lung metastases, 3 bone metastases and 3 local recurrence. The rate of CDF survival is slightly higher in good responders (50/68 - 74%) than in poor responders (9/16 - 56%), but the difference is not statistically significant. Local recurrences were seen in 3 good responders who underwent limb salvage, but with intralesional surgical margins. The average time to relapse is 27 months (3-72 months). The 6-years overall survival is 80%.

The Authors conclude that today with neoadjuvant CT it is possible to cure more than 70% of patients with localized Oe and that in about 80% of cases amputation can be avoided.

O-64

TREATMENT OF HIGH GRADE OSTEOSARCOMA (OGS) WITH IFOSFAMIDE (IFOS), MESNA (MES), ADRIAMYCIN (ADR), HIGH DOSE METHOTREXATE (HD MTX) AND CISPLATIN (CDDP)

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102 patients (pts), 53 male and 49 female, median age 14 yrs (range 3-49) with high grade OGS were treated on a multicenter trial. 23 pts had metastases (mets) at diagnosis. 64 pts (63 %) had osteoblastic morphology, 12 chondroblastic, 9 fibroblastic, 2 telangiectatic, and 15 not otherwise specified. Sites were: distal femur 50; proximal tibia 17; proximal femur 6; proximal humerus 7; pelvis 7; other 15. Preop chemo was IFOS (1800 mg/m^2/day x5 days) with MES uroprotection and ADR (25 mg/m^2/day x3 days continuous infusion) at wks 0 and 5; MTX (12 gm/m^2 [max 20 gm] with leucovorin rescue) at wks 3, 4, 8, 9, 13, 14; and ADR and CDDP (120 mg/m^2) at wk 10. Surgery was at wk 15. Postop chemo was IFOS with ADR at wks 17 and 22; ADR with CDDP at wk 27; IFOS alone wks 32 and 37; CDDP alone wk 42; MTX wks 20, 21, 25, 26, 30, 31, 35, 36, 40, 41. Toxicity has included fever following 43% of ADR courses, acute reversible renal toxicity in 3 pts, transient acute encephalopathy in 3 pts, mucositis in 20% of courses, and 1 toxic death. 81% of pts had limb salvage procedures. Histologic response to preop chemo in 61 pts with non-metastatic (non-met) extremity tumors was 95-100% necrosis in 54% of pts; 90-94% in 7% of pts; and <90% in 39% of pts. For all pts in the trial, 52% had >90% necrosis. With a median follow-up of 30 months, the EFS and S for pts with non-met extremity tumors (N=70) are 78% and 94%, respectively. For pts with non-met, non-extremity lesions (N=9), the EFS and S at 30 months are 50% and 56%, respectively. In pts with mets at diagnosis, the EFS and S at 30 months are 32% and 47%. Pts with non-met extremity OGS have an excellent outcome; however, new approaches are needed for pts with mets at diagnosis and for those with non-extremity primary sites.

O-65

MULTIDISCIPLINARY APPROACH IN OSTEOSARCOMA - 13-YEAR EXPERIENCE

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Between 1981 to 1994, 257 non metastatic osteosarcomas were treated with chemotherapy and surgery. There were 112 males and 145 females. Median age was 13 years (ranged, 3 ½ to 21 years). Primary sites were femur (51%), tibia (31%) and humerus (11%). 239 patients were evaluable for analysis in three different periods of treatment. From 1982 to 86, 84 patients were treated with Cisplatin (CDP) and Adriamycin (ADR), 1987 to 90, 92 patients with high dose Methotrexate -Lecovorin (MTX) containing regimen and between 1991 to 1994, 63 patients with CDP, ADR, Ifosfamide (IFOS) and Etoposide (VP-16) without MTX.

Surgery was performed in 237 patients: ablative in 47% and conservative in 53%. Overall survival and disease free survival were 42% with median time of follow up of 7 years. All relapses occured within 36 months of diagnosis.

Results concerning different periods are as follows in this table:

Period (year)	82-86	87-90	91-94
n° of patients	82	92	63
ablative surgery	49 (68%)	36 (41%)	11 (18%)
conservative surgery	23 (32%)	51 (59%)	50 (82%)
necrosis grade III+IV	22 (44%)	33 (46%)	45 (79%)
overall survival	47%	55%	82%
disease free survival	43%	53%	80%

In conclusion: chemotherapy and adequate surgery improve cure rate for patients with osteosarcoma. Agressive multiagent pre-operative chemotherapy increases the number of limb salvage procedures. Grade of necrosis has an impact in disease free survival.

O-66

A PILOT STUDY OF PREOPERATIVE CHEMOTHERAPY WITH 5 DRUGS IN LOCALIZED OSTEOSARCOMA OF THE EXTREMITIES. (FRENCH PEDIATRIC ONCOLOGY SOCIETY)

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In an attempt to improve good histological responses to preoperative chemotherapy in osteosarcoma, we proposed a 5 drugs regimen before surgery in all new patients (pts) treated from March 1989 to July 1993.

Material and methods - 62 pts with localized osteosarcoma of an extremity were included in this multicentric pilot study. There were 39 males and 23 females, with a mean age of 14 years old. 44% of the primary localizations were on the distal femur and 26% on the proximal tibia. All the pts had a biopsy-proven osteosarcoma, a normal CT scan of the chest and a Tech^{99} bone scintigraphy and 94% had a local MRI.

The preoperative chemotherapy regimen consisted of : 7 courses of high dose methotrexate (HDM) and 2 courses of Vindisine, Adriamycin, Cisplatinum and Ifosfamid (Help-Adri) over 3 months. After surgery, the pts received either the same regimen or 4 courses of Help-Adri.

The preoperative regimen was changed in 14 cases : because of HDM toxicity in 7 cases and tumoral progression during the first 3 HDM in 7 cases. Those 14 pts were given 3 or 4 courses of Help-Adri. One patient refused further treatment after 3 HDM and 1 HelpAdri. Surgery was performed in 59 pts (2 parents refusal and one progression) with 8 radical surgeries and 51 conservative ones.

Toxicity - HDM toxicity is well known and was severe in 10% of the pts. Help-Adri toxicity was important but essentially hematological : febril neutropenia occured in 21% of the preoperative courses (n=139) with 6 documented infections (5 septicemia). We had no toxic death but the treatment was postponed because of toxicity in many cases. There was no renal, no cardiac damage, and no hearing loss.

Results - 38 pts (64%) achieved a good histologic response (GR), and 21 (38%) a bad one (BR), With a median follow-up of 57 months (30-80), the overall and progression free survival (PFS) is respectively 77% and 59 % at 5 years. This PFS is 61% in GR and 65% in BR.

The multivariate analysis performed determined that the only prognosis factor in this series was the primary femur localisation (p=0.04 and Relative Risk=5.4)

Conclusion - This regimen brings a high good histological response rate but it is very toxic. Of interest is the fact that bad histological responders progression free survival is not significally different from good ones.

O-67

LONG TERM RESULTS AND LATE SEQUELAE IN OSTEOSARCOMA (OS) PATIENTS (pts) TREATED WITH T10 PROTOCOL

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In 1981, there was strong controversy over the role of chemotherapy in the treatment of osteogenic sarcoma. The most successful approach in terms of disease-free survival was Rosen's T10 protocol. At that time we considered Rosen's results as a challenge and decided to adopt the T10 protocol as a whole. Between 04.1981 and 12.1986, 70 primary, non metastatic os pts entered this protocol ; median age was 12 years ; conservative surgery was performed in 57 pts and amputation in 13. The histological response was good in 39 pts and poor in 31 pts. With a median follow up of 70 months DFS is 68 % at 6 y ; 75 % for good responders and 60 % for bad responders. Overall survival is 72 %.

Evaluation of late sequelae is currently performed in survivors 4 to 14 y after diagnosis (median 9 y). **Neurological evaluation** : 23/26 brain CTS are normal. In 3 cases hypodensity of the white matter is observed, associated to calcifications of the basal ganglia in 1 case. These 3 pts have no clinical disorders. **Renal evaluation** : 26 pts had a creatinine clearance dosage, 13 of them having received Cisplatinum. A moderate diminution is observed in 2 cases.

Hepatic evaluation : 1/33 enzymatic dosage is elevated (x20) due to a chronic Hepatitis C. 19 pts had viral serologies : 2 are positive for Hepatitis C and none for HIV. **Auditive tests** are altered in the high frequencies in 6 pts who received CDDP. **Cardiac Echography** was performed in 21 pts : 3 had a RF < 25 % and receive a medical treatment ; 2 have a major cardiac failure and are waiting for a heart graft. **Fertility** : 3/21 boys have an elevation of FSH. 5 pts (2 girls and 3 boys) have children.

These results confirm the excellent survival of OS pts treated according to T10 protocol as published by Rosen.

The major late effect is the anthracyclin related cardiac toxicity. Fertility seems to be preserved in males.

O-68

CHEMOTHERAPY,METASTASECTOMY, AND *LIPOSOME MURAMYL TRIPEPTIDE TRIPHOSPHATIDYL ETHANOLAMINE* (MTP-PE) FOR PULMONARY METASTASES IN PEDIATRIC OSTEOSARCOMA

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The primary tumor in 132 patients (pts) was treated with intra-arterial cis-diamminedichloroplatinum II followed by surgical extirpation. Postoperatively, high-dose methotrexate with leucovorin rescue, Adriamycin, and cyclophosphamide as stipulated by protocol and extent of tumor destruction were administered. Pts who presented with, or developed, pulmonary metastases underwent metastasectomy in the absence of tumor eradication by chemotherapy. Postoperatively Ifosfamide (all pts) and MTP-PE (6 pts) were administered. Pulmonary metastases were identified in 53 pts (13 at presentation): unilateral 21, and bilateral in 32. Thirty-four pts had lesions considered amenable to surgical resection. 19 unilateral and 15 bilateral (criteria will be presented). These pts underwent one or more resections for tumor extirpation yielding a total of 84 operations. Nineteen pts not considered candidates for resection were treated with aggressive chemotherapy. The age of the pts undergoing operation ranged from 2 to 17 years (mean 13.6 ± 3.2 years). This was not significantly different from the nonoperative (chemotherapy) group (13.6 ± 4.0 years). Responses to chemotherapy were achieved but were not durable. The number of metastases in the operative group ranged from 1 to 7 (mean 2.3). Of the 84 operations, 74 comprised wide resection, lobectomy 5, pneumonectomy 2 and wide resection plus chest wall resection 3. In over 90% of pts, tumor free margins were achieved. Survival was 8 of 53 (15%). Eight of 34 pts (24%) subjected to metastatectomy, chemotherapy and MTP-PE were alive and well 7.9 ± 2.0 years after resection (range 3.4 to 10.3 years). Cure was only achieved in patients subjected to metastasectomy. Chemotherapy and MTP-PE, by reducing the number of metastases and delaying their appearance could possibly have facilitated the procedure.

O-69

EARLY INTENSIFICATION WITH HIGH-DOSE THERAPY ELEMENTS DID NOT IMPROVE OUTCOME FOR HIGH-RISK ALL IN TRIAL ALL-BFM 90*.

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In trial ALL-BFM 83, poor response to a seven-day prednisone pretreatment and one IT MTX injection (PRED-PR) was identified as a novel variable to define ALL with very high risk (HR) of relapse. PRED-PR has since then successfully been used to identify the vast majority of HR-ALL in trials ALL-BFM 86 and 90. Probability of event-free survival (pEFS) for HR pts has been 0.46 (±.05) in trial '86. To further reduce recurrences among HR pts earlier treatment intensification was attempted in trial ALL-BFM 90 by applying three different six-day elements utilizing DEXA, TG, 6-MP, HD-MTX, HD-ARA-C, DOX, VDS, VCR, IFO, L-ASP, VP-16, and triple drug IT (TIT) immediately after induction in monthly intervals. This was followed by cranial irradiation (CRT) with 12 Gy (CNS disease at diagnosis: 24 Gy) before maintenance therapy was started. Pts. with PRED-PR *plus* an additional HR feature such as myeloid marker coexpression, high leukemic cell mass (BFM-RF ≥1.7), T-ALL, or pre-pre-B ALL, and pts with t(9;22), t(4;11), or nonresponse to induction therapy were qualified for allogeneic BMT in 1st CR.

After a median follow-up of 3 yrs, pEFS is 0.39 (±.04) for the 245 HR pts (=11.3% of all pts) enrolled in trial ALL-BFM 90 thus being slightly inferior to that of HR pts in trial '86 (p=0.24). A higher rate of isolated and combined systemic relapses is reflected by the probability of relapse-free interval (pRFI): 0.48 (±.04) in trial '90 compared to 0.64 (±.05) in trial '86 (p=0.01). However, despite reduced CRT, relapses with CNS involvement (3.5%) were now less frequent (p<0.01) than in trial '86 (12.6%). Pts qualified for BMT still fared poorly: pEFS=0.36 (0.34 in trial '86). In conclusion, early therapy intensification for HR pts in trial ALL-BFM 90 utilizing high-dose elements in monthly intervals could not prevent the high rate of systemic recurrences but a significant improvement in prevention of CNS relapses was achieved.

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O-70

MULTIDRUG RESISTANCE IN ACUTE LYMPHOBLASTIC LEUKEMIA IS RELATED TO *LRP BUT NOT TO MRP OR P-GLYCOPROTEIN EXPRESSION*

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In earlier studies we showed that in vitro drug resistance is a strong and independent prognostic factor in childhood ALL. This was found for several drugs including anthracyclines and vinca-alkaloids. In the clinical situation and especially in ALL, drug resistance depends on many different mechanisms for many different drugs. It has not been established which mechanisms of multidrug resistance (MDR) involving the anthracyclines, vinca-alkaloids and podophyllotoxins, are of clinical relevance in childhood ALL. This question was the topic of this study. ***Methods***: In vitro drug cytotoxicity was determined with the MTT assay. The expression of the MDR proteins P-glycoprotein (Pgp), multidrug resistance related protein (MRP) and lung resistance related protein (LRP) was studied by flow cytometry and immunocytochemistry using the antibodies MRK16 and C219 (Pgp), MRPm6 and MRPr1 (MRP) and LRP56 (LRP). ***Results***: Pgp and MRP expression were not different between untreated (n = 27) and relapsed (n = 15) ALL patients. Pgp and MRP expression were not related to DNR cytotoxicity nor to the combined score of cytotoxicity of DNR, VCR and VP16. In contrast to these findings, LRP expression was significantly higher in relapsed ALL (n = 16) than in untreated ALL (n = 29) (p = .005). Moreover, LRP expression was significantly related to in vitro resistance to DNR, VCR and VP16 (p = .015). ***Conclusion***: The data suggest that LRP but not Pgp or MRP is a clinically relevant MDR protein in childhood ALL.

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O-71

INDUCTION FAILURE AND EARLY RELAPSE OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA CAN BE PREDICTED BY IN VITRO DRUG SENSITIVITY TEST

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We studied the relation between in vitro sensitivity and clinical outcomes in 196 children with newly diagnosed ALL. We also explored how sensitivity to the combination of 4 drugs DPAV (Dex, Pred, Asp, VCR) predicted prognostic information, especially induction failure and early relapse (IF/e.rel, relapse within 180 days). **[Patients and Methods]** Eligible were children (age 0–16 years) with non–B ALL, newly diagnosed between 1989 and 1995. BM samples were sent for in vitro drug tests. There were 142 samples of common ALL, 21 of T–ALL, 28 of mixed lineage ALL (two or more positive out of CD13, 14, 15, 33), and 5 of undifferentiated ALL. All patients were treated with the ALL protocol of Pred, Asp, VCR and CPA or DNR as induction therapy. In vitro tests were carried out with a 4 day culture and MTT assay. We tested 16 drugs and calculated LD70 for 14 drugs, and LCS for Dex and Pred. For each single drug, patients were classified into two groups, as S (lower than median LD70 or LCS) or R (higher than median LD70 or LCS). **[Statistics]** Kaplan–Meier method for EFS, log rank test, post hoc test (Fisher's PLSD), and contigency table analysis for multivariate comparison were conducted using StatView–J4.5 (Abacus Concepts). **[Results]** EFS (3 yrs) of pts with cALL, T–ALL, mixALL, uALL were 0.727, 0.560, 0.534, 0.600 respectively. S group pts of Pred, VP16, VCR and MIT had superior EFS to R group pts ($p<0.05$). When we classified into three groups (S, I, R) by sensitivity to 4 drugs (DPAV), EFS (3 yrs) of S group (n=41) was 0.833, that of I (n=79) was 0.752, that of R (n=75) was 0.546 ($p=0.0011$). Then we investigated if drug sensitivity of S or R was related to three prognostic groups (CCR, IF/e.rel, late relapse). S groups of Pred, BLM, VP16, VCR, MIT were significantly related to CCR, and R to IF/e.rel ($p<0.05$). When we used S, I and R for DPAV sensitivity, S and I pts tended to undergo CCR and R pts increased the risk of IF/e. rel and late relapse ($p=0.0034$). **[Conclusion]** In vitro drug sensitivity testing together with immunological marker testing provides prognostic information in childhood ALL.

O-72

CAN NON-RADIOACTIVE META-IODOBENZYLGUANIDINE (MIBG) RESTORE RESISTANCE TO GLUCOCORTICOIDS IN LYMPHOBLASTIC LEUKEMIA?

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Glucocorticoids (GC) such as prednisolone and dexamethasone are amongst the most active drugs used in ALL and it has been shown that both in vivo and in vitro resistance to GC are important adverse prognostic factors in ALL. Therefore, restoration or circumvention of GC resistance is a highly important issue but unfortunately not many studies have addressed this issue. Smets et al reported that MIBG in its non radio-iodinated form restored GC resistance in a resistant leukemic cell line (Leuk Res 1988). A possible explanation for this was an increased number of GC binding sites and an increased affinity of these receptors for GC. We therefore performed a pilot study in which cells from children with ALL and AML were incubated in vitro with different concentrations of MIBG and prednisolone alone and in combination for 4 days. ***Results***: A dose-dependent leukemic cell kill was observed for MIBG alone from 6 ± 8% at 1.25 μg/ml MIBG to 81 ± 17% at 40 μg/ml MIBG. This concentration range covers the clinically achievable concentrations of MIBG. In the 18 patients at least additive leukemic cell kills were observed for MIBG in combination with prednisolone: In 15 patients (10 ALL and 5 AML) additive cytotoxic effects of MIBG and prednisolone were detected. In the other 3 patients, i.e. 3 relapsed ALL cases with the highest level of GC resistance, a synergistic effect of MIBG and prednisolone was observed. The cell kill by 50 μg/ml prednisolone was potentiated by 10 μg/ml MIBG from 35% to 61%, from 34% to 60% and from 12% to 48% respectively in these 3 cases after correction for the cell kill of MIBG alone. ***Conclusion***: MIBG gives additive antileukemic effects in combination with prednisolone in all leukemic samples studied. In some GC resistant cases it gives synergistis antileukemic effects thereby partly restoring the GC resistance. Further in vitro and in vivo studies of these antileukemic effects of MIBG and possible GC resistance restoring effects of MIBG are warranted.
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O-73

TREATMENT FOR RELAPSES OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND NON B-CELL NON-HODGKIN LYMPHOMA (NHL) : A PILOT STUDY

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Patients and Methods : All children presenting with any first relapse of ALL or non-B cell NHL were eligible for the study. Three groups of patients were defined :
Group 1 (G1) : late (CR1>18 months) isolated or combined bone marrow relapse (BMR) of common ALL.
Group 2 (G2) : early (CR1<18 months) isolated or combined BMR of common ALL, any relapse of either T or "Very High Risk."ALL [t(9;22), t(4;11) or resistance to prednisolone prephase].
Group 3 (G3) : isolated extramedullary relapse of c-ALL.
For G1, achievement of CR2 was attempted with a combination of 3 blocks "R1-R2-R3" (modified according to BFM protocol). In case of matched sibling donor, BMT was recommended, otherwise, children received 2 subsequent cycles of "R1-R2-R3", followed by a maintenance therapy during 2 years (6-TG and MTX, with R3 every 3 months during the first year). Patients of G2 received two courses of "VANDA" (including cytosine-arabinoside 8 g/m² total dose (td), mitoxantrone 16 mg/m² td, VP16 450 mg/m², dexamethasone 100 mg/m², asparaginase 40 000 U/m²). In case of matched sibling or unrelated donor (MUD), BMT was indicated. Other children were given a sequence "COPAdM" followed either by a intra-familial (IF) mismatched (MM) BMT or an autologous BMT (ABMT). Treatment scheme for G3 consisted in two cycles "R1-R2-R3" followed by a maintenance therapy (6TG + MTX), during 12 months and a local radiothcrapy.
Results: Between 5/90 and 12/95, 174 children were enrolled in the study : 65 in G1, 83 in G2, and 26 in G3. In G1, CR2 was achieved for 61 patients (94 %). Among the 59 evaluable children, 14 underwent BMT and 45 received chemotherapy (CT). In the BMT group, 10 children are alive in CR2 with a median follow up (FU) of 18 months (2-49) ; 3 relapsed and 1 died of HUS. In the CT group, 28 children are alive in CR2 with a similar FU and 16 experienced a relapse. The 2-year Event-Free Survival (EFS) for group 1 was 56 % (CI 41-70%) and 68 % (CI 37-88 %) and 54 % (CI 36-71 %) for BMT and CT group respectively (log rank p = 0,19). In G2, CR2 was achieved for 64 patients (77 %). Of those, 17 didn't undergo a BMT because of relapse (n=11), or toxicity (n=3) or insufficient follow-up (n=3). Six children underwent a BMT out of protocol after a second relapse. The 41 remaining children underwent BMT, an AMBT (n=13), a geno-identical BMT (n=13), a pheno-identical BMT (n = 6), a mismatched IF BMT (n = 9).

type of BMT	Number	Alive in CR2	Relapse	Toxic death
Autologous	13	10	3	0
Geno-identical	13	7	5	1
Pheno-identical	6	2	1	3
Mismatched	9	2	2	5

The 2-year EFS in G2 was 26 % (CI 17-38 %) and 46 % (CI 20-62 %) for the 41 grafted children in CR2. In G3, CR2 was achieved in the 25 evaluable patients (100 %). Two children were censored because of protocol violation. Among the 23 remaining patients a toxic death occured in 2 and subsequent relapse was observed in 7. Fourteen children are alive in CR2 with a median FU of 17 months. The 2-year EFS was 44 % (CI 24-67 %).
Conclusion : Encouraging results were obtained in G1, either after BMT or CT, but larger series and longer FU are needed to confirm these preliminary results. In G2, BMT is an effective consolidation treatment as almost half of these patients are probably cured.

O-74

EFFECTIVE TREATMENT OF REFRACTORY AND RELAPSED ACUTE MYELOGENOUS LEUKEMIA OF CHILDHOOD WITH IDA-FLAG (IDARUBICIN, FLUDARABINE, HIGH-DOSE CYTARABINE, GRANULOCYTE COLONY-STIMULATING FACTOR)

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Intensive chemotherapy has improved the prognosis of patients with AML. The success rate of relapse treatment correlates with the length of first remission. Thus early relapses and primarily refractory diseases have a grave prognosis. New chemotherapeutic regimens could be useful for those patients. Patients treated for refractory AML or AML relapsed within 2 up to 31 months after the first CR entered a pilot trial, the so called IDA-FLAG regimen. This regimen includes G-CSF (day 0 up to ANC > 1000/μl, 400 μg/m².d), fludarabine (day 1 - 4, 30 mg/m².d), high-dose cytarabine (day 1 - 4, 2000 mg/m².d) and idarubicin (day 2 - 4, 12 mg/m².d). 10 patients aged 1,8 to 28,1 years (mean 9,6 years) having the first (n = 8) or second relapse (n = 1) of AML or an acute blast crisis of myelodysplastic syndrome (n = 1) (FAB classification: M1/M2 = 3, M4/M5 = 5, M7 = 1, CMML = 1) received 14 courses.
Overall, 7 patients achieved CR with a mean duration of 10,0 months (1-24 months), one patient showed a partial remission and two were nonresponders. 4 patients are in continuous CR for 9,5 to 24 months (mean = 15,2 months). 3 patients got a bone marrow transplantation (allogenic = 2, autologous = 1) in CR following this treatment. Toxicity was considerable, mainly bone marrow aplasia with leucopenia <1000/μl for 15 to 40 days (mean 26,1 days), neutropenia < 500/μl for 14 to 39 days (mean 26,0 days) and thrombocytopenia < 30000/μl for 14 to 90 days (mean 36,5 days). Further important side effects were fever, mucositis and pneumonia. One patient died from an fulminant aspergillus sepsis during long-term neutropenia.
The IDA-FLAG regimen is effective in the treatment of relapsed or refractory AML of childhood and an advisable option prior to allogenic or autologous bone marrow transplantation. With regard to the unfavorable prognosis of relapsed or refractory AML the toxicity of this regimen seems acceptable.

O-75

A REVIEW OF THE CASES OF PAEDIATRIC MYELODYSPLASIA NOTIFIED TO THE UK REGISTER *1990-1995*

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The UK paediatric MDS register has 65 patients diagnosed, reviewed and verified centrally since January 1990. There were 19 cases of RA, 1 with RARS, 11 with RAEB or RAEBt, 14 with JCML, 7 with infantile monosomy 7 (Imo7), 12 with CMML (excluding JCML and Imo7) and 1 with Eosinophilia associated with MDS. Cytogenetic analysis of the bone marrow was available for 62 of which 35 showed an abnormality including 3 with constitutional abnormalities and one with increased spontaneous chromosomal breaks.
Treatment has been with bone marrow transplantation in 31 children, 16 from a matched family donor and 15 from an unrelated donor (UD). The amount and type of pretransplant chemotherapy has varied considerably. A further 16 children received intensive chemotherapy as for AML and four of these had autologous BMT (ABMT). The rest had no treatment n=11, oral drugs n=4, splenectomy n=1 and other n=2. Eleven of the children who had allogeneic BMT are alive 17-64 months from diagnosis compared with 3 of 14 who had UD BMT and 7 of 17 who had intensive chemotherapy with or without ABMT. Only one of the six children who had UD BMT for JCML survives and this child has chronic graft versus host disease. Half of the 12 children who had no treatment are alive. It was possible to allocate an "FPC" (based on HbF, platelet count and cytogenetics) score to 41 of the 65 children. Thirteen of the 22 with score 0 are alive, 5 of the 9 with score 1 and 1 of 12 with score 2.
The register has proved successful in its first five years and has enabled assessment of the various treatment options and the prognostic scoring system.

O-76

MYELODYSPLASTIC SYNDROME IN CHILDREN

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During the 4 years period twenty children with myelodysplastic syndrome (MDS) or 7 % of total number of the acute leukemias, have been diagnosed. The diagnosis of MDS was established on the basis of cytomorphology of the peripheral blood and bone marrow cells, histopathology and cytogenetic analysis of bone marrow. Cytogenetic anomalies were confirmed in 14/20 or 70% MDS : monosomy 7 in four or 20% , monosomy 19 in one , monosomy 13 in one, t(1:21) in one , anomalies of the chromosome 9 in two , t(1:9) with del(9) and inv(9). In six children the clone with hyperdiploidy (50-91 chromosomes), was settled, in one associated with del (13). Four of twenty children died because of CNS bleeding during the preleukemic phase. In five patients disease progressed into AML (Mo, M4 in two, M6 and M7). All children have been treated by the protocol BFM-87 with poor response and died. Eleven children are alive. In two with monosomy 7 and the diagnosis of RAEB and RAEB-t, allogenic bone marrow transplantation has been performed with successful outcome (30 mo follow up). Considering the rest nine patients, in six cytogenetic anomaly was confirmed , hyperdiploidy in four , monosomy 19 in one and t(1:9) with del(9) in one. Three of them have had histopathological features of myelofibrosis associated with hypoplastic MDS, two without cytogenetic anomaly until now , and one with hyperdiploidy. All children had cytopenias of different severity but only two have been transfusion dependent. HLA typisation was performed and further modalities of treatment were considered in the group of patients with clonal abnormality.

O-77

HEMATOLOGICAL FEATURES IN CHILDREN WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) ACCORDING TO KARYOTYPE

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We recently conducted a retrospective analysis of 110 children with CMML from Austria (n=9), Denmark (n=12), Germany (n=58), Italy (n=23) and The Netherlands (n=8). Patients (pts) were entered irrespective of the chromosomal aberration of the malignant clone, thus including patients with monosomy 7 (-7). Pts with -7 were recruited from every country except Italy. Data on the karyotype at diagnosis were available in 97/110 patients. Sixty-five pts (68%) had a normal karyotype, 18 pts (19%) -7 only, 4 pts (4%) -7 and additional abnormalities and 8 pts (8%) other abnormalities including 7q- in 4 pts. Pts with -7 only did not differ from those with normal karyotype with respect to age, sex, liver or spleen size, lymphadenopathy, skin infiltrates, presence of neurofibromatosis and survival. They did, however, display some characteristic hematological features. In the peripheral blood (PB) pts with -7 presented with a significantly lower white blood cell count (median 19.2 vs 36.6 x 10^9/L) and a higher percentage of monocytes (14.0 % vs 5.3%) while the absolute monocyte count did not differ from that observed in pts with normal karyotype. Red cells of pts with -7 were more often macrocytic (macrocytosis in 50% vs 16% of pts), while hemoglobin (Hb) concentration, reticulocyte and nucleated red cells count were similar. Pts with -7 had Hb F levels within or just above the normal range while a wide range of Hb F was noted in children with normal karyotype. In the bone marrow (BM) pts with -7 displayed more pronounced erythropoiesis (EP) (granulopoiesis/EP 1.7 vs 3.2) and a higher percentage of monocytes (17.7% vs 7.6%) and eosinophils (10.0% vs 3.8%). There were no significant differences between the two groups concerning the blast cell count in the PB or BM. Our data indicate that pts with CMML and -7 may not differ by clinical but by hematological criteria from those with normal karyotype.

O-78

MONOSOMY 7 IN MYELODYSPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML)

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Monosomy 7 is the most common cytogenetic abnormality in childhood MDS and has been considered a distinct hematologic disorder called infantile monosomy 7 syndrome. In a cooperative study of EWOG-MDS (Austria, Czech Republic, Denmark, Germany, Italy, and the Netherlands) we retrospectively reviewed 75 children with monosomy 7 and myeloid disorders. Classification was performed according to FAB criteria of MDS and AML. The morphologic diagnoses were MDS 51 (RA 5, RAEB 10, RAEB-T 7, CMML 29), AML 24 (M0 5, M1 5, M2 9, M4/M5 3, M6 2). Four patients had previously received chemotherapy. No cases were associated with prior G-CSF administration. Complete monosomy 7 was found in 59 (as the sole abnormality in 45). Deletion 7q occurred in 16 (9/24 AML, 6/29 CMML, but in only one of 22 with RA/RAEB/RAEB-T). The median age was 3.3 years (range 3 months - 18 years). Children with CMML had, compared with RA/RAEB/RAEB-T and AML, a higher hemoglobin (9.5 *vs* 8.0 g/dl), lower MCV (87 *vs* 94), higher WBC (20 *vs* 6 10^9/l), higher male:female ratio (2.2 *vs* 1.1), lower median age (13 *vs* 59 months), and presented more frequently with hepatomegaly (93 *vs* 54 %) and splenomegaly (89 *vs* 37 %). The 3-year survival was 56 % in those receiving BMT (n=31) *vs* 29 % in those not treated with BMT. AML induction therapy not followed by BMT was given to 29 patients with only few survivors (CMML: 0/7, RA/RAEB/RAEB-T: 2/7, AML: 5/15). Of those transplanted following AML therapy 4/14 survived *vs* 10/17 of those who had no induction chemotherapy prior to BMT. Monosomy 7 occurred in a heterogeneous group of myeloid disorders and our data give no support to the concept of monosomy 7 as a distinct syndrome.

O-79

THE ROLE OF INTENSIVE AML-SPECIFIC CHEMOTHERAPY IN TREATMENT OF CHILDREN WITH RAEB AND RAEB-T

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To determine the role of intensive chemotherapy in treatment of refractory anemia and excess of blasts (RAEB) or RAEB-T (in transformation), 39 children aged 0.9 - 17.2 years (median 8.2) and diagnosed between 1985 and 1995 were analysed. Blast count in the bone marrow at diagnosis varied between 7% and 30%, (median 16%). Karyotyping in 21 patients (pts) revealed abnormalities in 11 pts, with monosomy 7 in 4 of them. Thirteen pts received intensive chemotherapy according to the AML-BFM protocol 83, 87 or 93 (group1). Nine pts were treated less intensively with the 6-week consolidation phase as induction (group 2). 17 children did receive minimal or no chemotherapy (group 3). Allogeneic bone marrow transplantation (BMT) was performed in 4/13, 4/9 and 7/17 children of the respective groups. Results indicated that with chemotherapy only 8/13 children of group 1, 5/9 of group 2 and 0/17 of group 3 achieved remission. 5-year survival of the total group was 37%, SE 11%. 9/14 children receiving AML specific chemotherapy (group 1 and 2) were alive without, and 7/8 with BMT. Outcome after BMT was related to the blast count in the bone marrow prior to BMT. All 7 children (including 2 with minimal or no chemotherapy) with $\leq$ 10% blasts before BMT were in remission, whereas 5/8 patients with a higher blast count relapsed after BMT (p logrank 0.04). Feasibility of intensive treatment (duration and toxicity) was similar to that observed in children with AML. Our data support the conclusion that children with RAEB or RAEB-T can achieve remission with an intensive AML-specific chemotherapy. In pts responding to intensive chemotherapy an increase in long-term survival after allogeneic BMT can be expected.

O-80

Allogeneic BMT for chronic myelomonocytic leukemia (CMML) in childhood: a report from the European Working Group on Myelodysplastic Syndrome in Childhood (EWOG-MDS)

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Purpose: To evaluate in a large cohort of patients the role of BMT in the treatment of children with CMML and to analyze the impact of different patients and treatment-related variables on patients' outcome.

Patients and Methods: Forty-three pediatric patients with CMML given an allogeneic BMT and reported in the database of EWOG-MDS were evaluated. In 23 cases the donor was an HLA-identical sibling, in 6 cases a partially matched family donor (2 of whom with a 1-antigen disparity) and in 14 a matched unrelated donor (MUD). The conditioning regimens comprised total body irradiation (TBI) and chemotherapy in 22 patients, whereas busulfan (Bu) with other cytotoxic drugs was used in the remaining 21 patients.

Results: Six of the 43 patients (14%), 5 of whom receiving BMT from alternative donors, failed to engraft. In the other 35 patients the median time to achieve granulocyte and platelet recovery was 19 and 29 days, respectively. Grade II-IV GVHD occurred in 33% of children transplanted from HLA-identical siblings or 1-antigen mismatched relatives and in 62% of children given BMT from other donors. Probability of transplant-related mortality was 20% for the entire group, 9% for children given BMT from HLA-identical/1-antigen disparate relatives and 46% for patients transplanted using MUD or mismatched relatives. The actuarial probability of relapse for the entire group was 58%, whereas the 5-year product limit EFS was 31%. The EFS for children given BMT from an HLA-identical sibling or a 1-antigen disparate relative was 38%. In this latter group, patients receiving Bu had a significantly better EFS in comparison to those given TBI (62% vs 11%, P<0.01).

Conclusion: Children with CMML and an HLA-compatible family donor should be offered allogeneic BMT as early as possible or, at least, before they have presented disease progression. Improvement of donor selection, GVHD prophylaxis and supportive care are needed to ameliorate results of BMT from alternative donors.

O-81

WHAT CHILDREN'S DRAWINGS TEACH US ABOUT THEIR EXPERIENCE OF CANCER

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Children treated for cancer draw profusely. During psychotherapeutic interviews, their comments allow us to gain access to the complex, subjective experience they are going through. Eight drawings are presented. 1- The expression of extreme loneliness in a deserted world. 2- The fear that treatment will lead to death even though it is powerful. 3- Where does cancer come from ? In what way is my family responsable ? 4- A young girl expresses her doubts about becoming a woman, a mother. 5-Will radiotherapy cure or kill me ? 6- The world has become a machine and I am locked inside. 7- The experience of cancer has split my body and my family into two parts. 8- Despite my medical recovery, the experience of cancer is not over and remains a gaping wound. Every detail is important in the drawings : their beauty, the intense emotion they transmit, their themes, colours, style, their gradual elaboration and, most of all, the child's comments and associations of conscious and unconscious thoughts. In a series of interviews, the development of each element must be given undivided attention. More than signs of psychological and neurological illness, it is the child's way of transmitting to us, with total confidence, the precious elements of his experience, imploring our help so that his/her perception of his/her own life remains personal and is not distorted.

O-82

HOW TO PRACTICALLY ASSIST A DYING CHILD FROM LEUKEMIA: A "WINNING APPROACH" !

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Although there is now an increasing interest to evaluate different approaches to children with end-stage neoplastic disease, relatively few studies have focused their attention on children with leukemia. We report the experience of our center in the last 4 years (1992-'95) about the modalities of approaching the dying child from leukemia and his family. **Main goal:** to obtain the best "quality of life" of both, child and family, through a supportive and palliative care based on the control of physical and psychic pain. **Patients:** We considered 40 children: 25 male, 15 female, 29 (72.5%) affected by acute lymphoblastic leukemia (ALL) and 11 (27.5%) by acute non lymphoblastic leukemia (ANLL). Mean age was 6.5 yrs at diagnosis (range: birth-15 yrs) and 9.7 yrs at death (range 9 months -18 yrs). The terminal phase (defined as no longer responding to chemotherapy or with multiple relapses and no possibility of cure) lasted for a mean of 51.5 days (range 2-128) in 35 children and 271 days (range 144-519) in 5 children with a so-called "slow" disease. **Methodology:** The quality of life of children was evaluated through: a) "Play-performance scale" by J.B. Lansky b) Their school attendance c) The meeting with parents after the death of their child d) Pain control e) Place of death f) Family doctor's role. **Results:** a) 29/40 children (72.5%) obtained 80-100 points (=normal to quite normal daily activity) b) 19/28 (67.8%) attended school quite regularly (at least 4 times/week until few days from death) c) 36/40 (90%) families met the physician after the child's death d) in 35/40 (87.5%) the pain was considered adequately controlled. Drugs administered: morphine p.o. + Benzodiazepines e) 25/40 (62.5%) children died at home f) in 30 cases the family's doctor was successfully involved (the majority of their children died at home). The **"winning approach":** seven gold-rules: 1. A physician as principal coordinator of a well structured program 2. Presence of at least one nurse particularly involved and devoted 3. Another physician chosen with the family 4. The family-doctor involved since the beginning of the disease 5. Continue the periodical controls in the clinic 6. Frequent phone calls to encourage the family 7. Adequate and easily applicable antipain medications.

O-83

MAKING SCHOOL A SUCCESS FOR THE CHILD WITH CANCER, AND THEIR SIBLINGS

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Studies and experiance tell us that children with cancer may develop school phobia due to a) falling behind scholastically, b) social isolation, c) teasing and d) lack of teacher understanding. In striving to give the child with cancer a full life, we recognize the importance of school as a normalizing agent. In order to assure this, we offer the following service: 1) Daily schooling by hospital teacher during treatment, 2) Follow-up by home-school teacher while isolated in the home, 3) Information-visit to the child's school (and the sibling's) by the primary oncology nurse. The information is given at a teacher staff meeting (with parents present) and in the children's classrooms (with the child present)
At the staff meeting, the purpose is to help them understand the medical, emotional and social impact of the illness on the family as a whole, and to provide a personal link to the medical team.
In the classroom the emphasis is placed on accurate, easy-to-understand information focusing on the child's/sibling's situation and then discussing illness and hospital etc. in general.
An afternoon spent at school, by the oncology nurse, has turned out to be a sound investment in the child's rehabilitation.

O-84

THE DIAGNOSIS OF OSTEOSARCOMA: AN OVERWHELMING EXPERIENCE?

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The cure of a serious disease is never simple. For children with cancer end of treatment doesn't necessarily mean relief and tranquillity - once treatment is completed, they have to continue their lives now with multiple and complex difficulties.
Based on psychoanalytic approach, 34 patients treated of osteosarcoma were interviewed to study the psychological process of recovery. Interviews addressed physical, intellectual, affective, school, professional, familial and social aspects. All patients were at least 12 months off therapy (mean 58 months). 15 were boys and 19 girls. Mean age at diagnosis was 13.6 years (range from 7 to 19 years) and mean age at interview was 18.4 years (range from 9 to 25 years). 22 patients underwent limb salvage procedures and 12 ablative surgeries.
Analysis of interviews showed that subjective experience of recovery doesn't coincide with end of treatment - some elements of the disease and treatment continue to affect for months, even years later, patients emotionally in spite of the site of the tumor, age at diagnosis, time off therapy or physical sequelae. Cancer history persists throughout their lives as an insuperable model, as an obligatory reference.
In summary, it is essential to identify early signs of emotional difficulties beginning at the time of diagnosis, continuing during treatment and recovery to define the interventions that will help patients to overcome this intense experience without late secondary effects

O-85

STANDARDS OF PSYCHOSOCIAL CARE IN PEDIATRIC ONCOLOGY

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Bridging the gap beteen science and practice in pediatric oncology psychosocial care is needed, if our discipline attempts to utilize more strictly what is known about high quality psychosocial care. A new discipline - Versorgungspsychologie* (care-psychology) - is concerned with the realisation of achievable high quality standards. The task is to develop standards of psychosocial care, and to test them under conditions of everyday practice.

Method: Since July 1994 30 patients and their families were cared for according to a psychosocial protocol. Intervention-orientated questionnaires were given to the patient, his/her family, the physicians and the nurses at special points in time during medical treatment. The data of the questionnaires build the basis of our "help for selfhelp". According to the concepts of "informed consent", "adherence" and "reappraisal/recovery" we have developed psychosocial oncology intervention strategies.

Results: The results show good coping in 60% of families. About 25% show some and 15% significant problems in coping. As an average, the nursing personal and the physicians rated aspects of informed content, adherence, and recovery of the families as being satisfactory, that is, patients and parents can help themself during treatment at a satisfactory level. The rate of satisfaction of the staff and the families with their personal situation on the ward varies from month to month between 56% and 95%.

Conclusion: Standardized psychosocial care in pediatric oncology is possible and successful. We can thus take care of all families according to their individual needs. The quality of teamwork on an oncological ward may be monitored and improved during the course of treatment.

* Kusch, M., Labouvie, H., Fleischhack, G. & Bode, U. (1996). Stationäre psychologische Betreuung in der Pädiatrie. Weinheim: Psychologie Verlags Union.

O-86

INFLUENCE OF RECULTIVATION-TIME ON THE MEASUREMENT OF CELLULAR RESISTANCE

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Several parameters influence the measurement of cellular resistance to cytotoxic treatment. We addressed the time between the end of the treatment and the determination of the cell number. The resistance after radiation or repeated incubation with cisplatinum (CDDP) was used as a model.

86HG-39, A172, T98G human glioma cells and TE671 human rhabdomyosarcoma cells were pretreated with either 9 Gy ^{60}Co gamma irradiation or 10^{-6} M CDDP for 24h. The sensitivity of pretreated cells was compared to control cells. 9 Gy or different concentrations of CDDP were applied and the cell number was determined using a colorimetric test system at 5 timepoints between 48h and 346h after the cytotoxic treatment.

72h after radiation 39% (±4.7 S.D.) of four times CDDP pretreated TE671 cells survived compared to 42% (±1.7) of the control cells indicating no resistance to radiation. However 264 h after radiation 50% (±2.1) of the pretreated cells were alive compared to 4.5% (±0.8) of the control cells indicating resistance to radiation ($p<0.0001$). Similar data were found with respect to resistance to CDDP and in several other cell lines: Only 4 of 29 experiments showed resistance in early but 19 in the late measurements. We conclude that experiments with early measurement might fail to show resistance and should be interpreted cautiously. Supported by Deutsche Krebshilfe.

O-87

IMBALANCE IN NUCLEOTIDE POOLS IN ALL DUE TO THE INCREASED ACTIVITY OF **CYTIDINE TRIPHOSPHATE SYNTHETASE.**

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Introduction: Patients with ALL possess altered nucleotide profiles in their lymphoblasts compared to resting lymphocytes, that resolve after achieving remission. The increased concentration of **cytidine triphosphate (CTP)** is the major aberration in the nucleotide pattern and is also present in leukemic cell lines. Through studying nucleotide fluxes in a MOLT-3 lymphoblastic cell line we showed that the increased CTP concentration is the result of an increased activity of **CTP synthetase** (CTPS), which is in line with the increased activity found in other malignancies. CTPS is the rate-limiting enzyme in the synthesis of CTP via the "de novo" pathway and the salvage of uridine. Therefore, CTPS provides an attractive target for a new chemotherapeutic approach.
Methods: Presented are the CTPS activities measured in lymphoblasts of 18 pediatric patients with ALL at diagnosis.
Results: The mean enzyme activity proved to be substantially higher in lymphoblasts compared to peripheral lymphocytes of healthy controls (11.3 versus 5.0 nmol CTP/mg protein/hr), which is correlated to the increased CTP concentration. Recently we have isolated the CD34/CD19 positive bonemarrow samples of controls in order to compare the enzyme activities.
Conclusion: Our results provide the first evidence that the CTPS activity is increased in lymphoblasts from children with ALL. Therefore, inhiting CTPS by a drug like **cyclopentenylcytosine** (CPEC) might be promising. CPEC is not only a potential cytostatic drug, but is also capable of enhancing the cytotoxic effect of arabinofuranosyl cytosine (Ara-C) in murine leukemia.

O-88

POTENT MODULATION OF P-GLYCOPROTEIN MEDIATED DRUG RESISTANCE BY GG918 IN MES-DX5 SARCOMA CELL LINES.

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Drug resistance to cytotoxic agents is a major obstacle for the successful treatment of malignancies. The membrane transporter P-glycoprotein confers multidrug resistance (MDR) by transporting cytotoxic drugs out of cells. This process can be reversed with MDR modulators like verapamil or cyclosporin A but their clinical use is restricted by low potency and high toxicity. The potency of the recently described MDR reversing agent GG918 (Hyafil, 1993, Cancer Research 53, 4595-4602) has been assessed. The resistance of the P-glycoprotein expressing human sarcoma cell line MES-Dx5 was assessed with a 72 hour cytotoxicity assay (MTT assay) and exhibited the following relative resistance factors to cytotoxics: etoposide 2, doxorubicin 6, vinblastine 12 and taxol 155 compared to the sensitive non-P-glycoprotein expressing MES-SA cell line. Full sensitivity of MES-Dx5 cells could be restored by GG918 with the following order of potency for GG918 to fully reverse drug resistance: vinblastine > etoposide > taxol > doxorubicin. The accumulation deficit of [^{14}C] doxorubicin, [^{3}H] taxol and [^{3}H] vinblastine in MES-Dx5 cells was fully reversed by 1uM GG918 and uptake reached 150% of baseline for all 3 cytotoxic drugs. Binding of [^{3}H] vinblastine to MES-Dx5 membrane fragments was examined. The saturation isotherm for [^{3}H] vinblastine revealed a B_{max} = 4.8 ± 3.4 pmol/mg protein and K_D = 11 ± 5 nM. The Ki values of drugs to inhibit [^{3}H] vinblastine binding were: doxorubicin 0.1 mM, verapamil 1.7 uM, taxol 96 nM and GG918 5 nM. GG918 was 235-fold more potent than verapamil in inhibiting [^{3}H] vinblastine binding to P-glycoprotein. *In vitro* the MDR reversing agent GG918 is modulating drug resistance potently because it binds tightly to P-glycoprotein.

O-89

ROLE OF PLOIDY, CHROMOSOME 1p AND SCHWANN CELLS IN THE MATURATION OF NEUROBLASTOMA.

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Ganglioneuromas are the mature counterparts of neuroblastomas (NB), the most common solid neoplasms in early childhood. These tumors, which are often found incidentally and behave clinically benign, consist of neuronal (neuroblastic/ganglionic) cells and Schwann cells which are thought to represent differentiated tumor cells. This assumption is mainly based on the presence of both cell types in maturing NBs and on in vitro-differentiation experiments using NB cell lines. Although genetic changes leading to aggressive NBs are well known, i.e. N-*myc* amplification, deletions at chromosome 1p36.3 and di-/tetraploidy, there was almost no information available concerning the chromosomal constellation of maturing NBs. We analyzed 25 maturing and mature NBs using in situ hyridization methods and flow cytometric measurement. Neuroblastic/ganglionic cells showed numeric chromosome aberrations indicating near-triploidy. The Schwann cells in all 25 NBs consistently had normal numbers of chromosomes. Chromosome 1 deletions were absent in both cell types. Based on this data, we conclude that i) near-triploidy and integrity of 1p are genetic prerequisites for maturation processes in NB and that ii) the NB-associated Schwann cells are normal cells, most likely attracted by NB cells. This assumption is also supported by the results we obtained from cocultivation of NB cells with Schwann cells. We therefore assume that the Schwann cells play a crucial role in the growth inhibition and differentiation of NB cells by expressing neurotrophins as it is well known from experiments carried out with non-neoplastic neuronal cells and Schwann cells. Our results provide a basis for a new access to the understanding of the maturation processes in neuroblastomas.

O-90

IMAGE AND FLOW CYTOMETRY IN DNA PLOIDY ANALYSIS FOR PROGNOSTIC ASSESSMENT IN NEUROBLASTOMA (NB): A REPORT ON 115 CHILDREN.

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DNA ploidy as measured by flow cytometry (FCM) has been shown to be useful for assessing prognosis in children with NB, but only in those younger than 24 mos. We previously showed that the accuracy of DNA content analysis can be increased by combining image cytometry (ICM) with FCM (Proc Am Assoc Cancer Res 1993;34:38). In the present study, samples obtained by needle or surgical biopsy from 115 children aged 1 day to 162 mos (median: 16 mos) at all INSS stages were analyzed. From each tumor, single-cell suspensions from frozen tissue were measured by FCM, whereas imprints from fresh or frozen tissue were measured by ICM. Ploidy was defined in terms of DNA index (DI). Based on our preliminary findings (ibidem), di-, tetra- and octaploid content was considered unfavorable, while tri-, penta- and hexaploid content was considered favorable. FCM and ICM findings were in agreement in 109/115 tumors ($p<0.001$). However, only ICM was able to discriminate cell populations with different ploidies within the same tumor, even when their number was below the FCM detection threshold. To this regard, we also analyzed 10 ganglioneuromas and all were diploid by FCM, whereas ICM identified a triploid cell population in most of these. Survival analysis data were available for 106 children. Forty died of disease and 66 (62%) were long-term survivors. According to ploidy as assessed by ICM, children with triploid (combined with penta- and hexaploid) NBs showed a better outcome in all age groups: 88% vs. 33% ($p<0.0001$). Pooling together diploid and tetraploid tumors made the prognostic significance of DNA content measurement more pronounced in children above 12 mos of age: in those aged 12 to 24 mos, p went from 0.1 to 0.01; and in those older than 24 mos, p went from 0.03 to 0.002 (73% vs. 17% survival). Because of its greater sensitivity in measuring DI values of different cell populations, even in imprints, ICM should be included in the prognostic work-up in children with NB.
Supported by the Swedish Cancer Society

O-91

GENETIC GAINS AND LOSSES IN NEUROBLASTOMA BY COMPARATIVE GENOMIC HYBRIDIZATION

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Neuroblastoma behavior is variable and outcome partially depends on genetic factors such as the number of DNA copies at particular sites in the genome. Tumors that lack bad prognostic factors such as NMYC amplification or 1p deletion may progress, possibly due to presently unknown genetic aberrations. Comparative genomic hybridization (CGH) summarizes DNA copy number abnormalities (CNAs) in a tumor by mapping them to their positions on normal metaphase chromosomes in a single experiment. We analyzed 69 tumors collected from the Children's Cancer Group and 3 French centers by CGH. There were 41 good prognosis (I, II, and IV-S) and 28 poor prognosis (III, IV) patients. The median and mode of the number of aberrations were 8 per tumor (range 0-15). Nineteen cases (27%) solely had CNAs of whole chromosomes, 8 cases (12%) solely exhibited CNAs on pieces of chromosomes, 33 cases (48%) had both whole and partial CNAs, and 9 cases (13%) had no CGH evidence of CNAs. High level amplifications were detected in 14 specimens (20%): at 2p23 (NMYC region) in 11, and 4q33-35, 5q32-34 and 6p11.2-22.1. 76% of cases had chromosome 17 gains: 28 had whole 17 gain and 24 cases had only 17q gain (17q+) - the common region was 17q21.3-qter. Other frequent gains were on chromosome 7 (58%), 6 (28%), and 18 (25%). Losses were frequent at 14q (33%), X (29%), 11 (26%), and 1p (25%). 1p deletion was found in 13 cases(46%) with poor prognosis and in 6 (15%) with good prognosis. 2p23 amplification was found in 11 (39%) with bad prognosis and only in 2 (5%) with good prognosis. The other 3 amplifications were in poor prognosis tumors. 17q+ was found more frequently in poor prognosis (16/28,57%) than in good prognosis disease (8/41,20%) (p=0.003). These results indicate that chromosome 17 aberrations occur commonly in all stages of neuroblastoma and may be an initiating event and that 17q+ is associated with bad prognosis disease. Supported by CA 61147 & 13525.

O-92

TEL/AML1 GENE FUSIONS IN CHILDREN WITH B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA: AN RT-PCR STUDY.

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Objective: Recently, the genes involved in a *TEL/AML1* fusion resulting from a cytogenetically undetectable translocation t(12;21) have been characterized. This study was undertaken in order to access the frequency of the TEL/AML1 rearrangement in a series of clinical samples.

Patients and methods: By RT-PCR we screened 240 randomly selected bone marrow samples from children with ALL at diagnosis of their disease. 215 cases were B-lineage in origin (pre-pre-B, n=8, CD19+,CD10-,CD20-,cyIgM-; common ALL, n=172, CD19+,CD10+, cyIgM-; pre-B, n=35, CD19+,CD10+/-, cyIgM+, surface IgM-), 19 patients had T-cell ALL, 6 cases were not classified.

Results: 231 cases were successfully investigated whereas the analysis failed in 9 cases due to RNA degradation. Out of 167 patients with common ALL and a sufficient RNA quality 12 (median age 3,5 yrs, 9 males, 3 females) were positive for a TEL/AML1 rearrangement. Cytogenetic results were available in 2 of the positive cases, one of them had a del(6q) and one had a normal karyotype. All TEL/AML1 positive cases had a good "prednisone response", but two of them had more than 100.000 leukocytes/µl at diagnosis. Nevertheless, none of the TEL/AML1 positive cases has relapsed to date (median follow up 2 yrs)

Conclusion: The TEL/AML1 rearrangement occurs in about of 7% of common ALL in childhood and seems to associated with a favorable prognosis. Based on these findings a prospective screening for the presence of TEL/AML1 in children with ALL of B-cell origin has been initiated within the ALL-BFM therapy trial.

O-93

T CELL PRIMING TO THE ACUTE LEUKEMIA FUSION PEPTIDE, MLL/AF-4, USING SYNTHETIC PEPTIDE PULSED SPLENIC LYMPHOCYTES

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The graft-versus-leukemia effect, after bone marrow transplantation, appears to be secondary to eradication of leukemia by reactive T cells *in vivo*. A potential endogenous leukemia-specific antigen which may be recognized by T cells is the fusion peptide product MLL/AF-4 secondary to the t(4;11) translocation in high risk acute leukemias. We evaluated whether T cell responses could be generated by immunization with i.v. administration of spleen cells pulsed with one of the MLL/AF-4 fusion peptides consisting of 10 amino acids on either side of a common breakpoint of t(4;11) found in infant ALL. Using the synthetic 20-mer peptide, GVHRIRVDFK/QTYSNEVHCV, we observed minimal proliferative T cell responses in BALB/c mice (H-2^d) and C57BL/6 (H-2^b). By contrast, significant proliferative responses of 5,550 ± 1,048 cpm was seen in lymph node derived whole population T cells from BALB.B (H-2^b) mice (mean ± SEM) and for H-2^k restricted T cell responses in AKR/J (10,559 ± 3,850 cpm) and B10.BR (4,930 ± 3,049 cpm) mice. Since the greatest proliferative T cell response was H-2^k restricted, we evaluated for induction of a cytolytic ($CD8^+$) T cell response in AKR/J mice. We were unable to induce a significant cytolytic response restricted to H-2^k target cells pulsed with any of the 9-mer peptides which are derived from the immunizing 20-mer. Thus, we were able to induce a significant proliferative T cell response to the MLL/AF-4 fusion peptide by *in vivo* T cell priming in mice. These data support further investigations to evaluate whether synthetic peptides may be used to activate either $CD4^+$ or $CD8^+$ T cell in humans as either a vaccine or as immunotherapy of acute leukemia.

O-94

INFANT ACUTE LYMPHOBLASTIC LEUKEMIA WITH t(4;11): EXPRESSION OF MULTIPLE MLL/AF4 TRANSCRIPT VARIANTS WITH USE OF CRYPTIC SPLICE SITES

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Molecular studies of der(11) transcripts of the MLL/AF4 fusion gene were performed in 10 infants with t(4;11)(q21;q23) positive acute lymphoblastic leukemia (ALL). Variants of the MLL/AF4 fusion message were investigated by reverse transcription-polymerase chain reaction (RT-PCR) and sequence analysis of cloned amplification products. To facilitate detection of splice variants expressed at a low level, the most abundant MLL/AF4 fusion products were selectively digested with restriction enzymes prior to amplification by PCR.

Molecular analysis revealed co-expression of multiple MLL/AF4 mRNA variants in 6 cases. In addition to previously described chimeric products with fusion of MLL exons 5, 6, 7 or 8 to AF4 exons A, B or C, fragments representing novel splice variants were observed. The alternatively spliced fusion transcripts resulted from exon skipping and use of cryptic splice sites. Interestingly, patients who showed activation of cryptic donor sites in MLL exon 5 did not exhibit any correctly spliced MLL/AF4 fusion transcripts. To assess whether the use of normal splice sites was prevented by mutation within these loci, the MLL exon 5/intron junction was investigated in three patients by SSCP analysis and specific digestion with Hph I which recognizes the junction sequence (G/GTGAG). No abnormality has been detected, suggesting that other mechanisms are involved in the selection of alternative splice sites. Usage of cryptic splicing sites occured in the MLL part of the fusion transcript in most instances. Only one case displayed an activated cryptic acceptor site in exon B of the AF4 gene resulting in a variant with open reading frame. Further analysis of the relationship between the course of disease and expression levels of heterogeneous der(11) MLL/AF4 transcripts is in progress.

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O-95

LEUKEMIA-ASSOCIATED PHENOTYPIC ABERRATIONS AND THE STUDY OF RESIDUAL DISEASE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA.

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The detection of minimal residual disease (MRD) in leukemia may have profound impact in the clinical management strategies. Forthcoming knowledge of the genetic and immunphenotypic properties and abnormalities of leukemic cells provides a basis for several assay systems. In a limited series of cases, the value of immunphenotypic analyses for identifying patients with impending relapse has already been established. However, in B-lineage acute lymphoblastic leukemia (ALL), possibilities for investigation were largely confined to a minority of cases characterized by myeloid marker coexpression. To develop new strategies for the investigation of B-lineage ALL patients, we investigated samples of normal pediatric bone marrow (n=40) as well as of B-precursor ALL at diagnosis (n=57) using 3-color multiparameter flow cytometric analysis. We assessed the complex phenotypic patterns of normal and leukemic B-precursor cells regarding qualitative and quantitative differences of expression of a large panel of antigens. Comparing to normal, leukemic samples displayed individual aberrations in the expression of certain antigens in a considerable proportion of cases. Overexpression of CD10 was displayed by 34 of 51 samples (67%). Independently, aberrant expression of CD45RA was found in 28 of 47 (60%), and of CD11a and CD44 in approximately one third of cases. In a pilot study, multiparameter analysis was applied to investigate bone marrow samples (n=44) of pediatric B-lineage ALL patients (n=34) during continuation therapy or off treatment. In 13 samples residual leukemic cells could be detected. All of these patients relapsed subsequently, the longest duration from analysis to overt disease being 9 months. In view of the data, we were able to define leukemia-specific phenotypic aberrancies in the majority of B-precursor ALL cases. Applied to the detection of MRD, this multiparameter flow cytometric approach proved to succesfully address patients with impending relapse. The procedure, therefore, promises to improve the diagnostic repertoir for the follow up of leukemia patients. An extended prospective evaluation is warranted to determine the sensitivity and specificity of the method at large.

O-96

***TEL-AML1* FUSION RNA AS A NEW TARGET TO DETECT MINIMAL RESIDUAL DISEASE (MRD) IN CHILHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCP-ALL).**

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A new cryptic translocation, t(12;21)(p13;q22), has been shown to fuse two genes, *TEL* on chromosome 12 and *AML1* on chromosome 21. We have evaluated its frequency in childhood ALL and the feasability of MRD monitoring by amplification of *TEL-AML1* anscripts. From June 93 to January 96, 96 children with ALL were diagnosed in our center (T-ALL: 14, BCP-ALL: 82). Out of 68 evaluable BCP ALL, *TEL-AML1* transcripts were identified in 16 cases (23,5%) by RT-PCR assay followed by hybridization of amplification products to a junction-specific oligoprobe (RT-PCR-JSO). This assay was negative in all T-ALL cases. A *TEL* rearrangement was demonstrated by genomic Southern-blot in 7/7 analysed cases. All but one children were aged between 2 and 10. There was no statistically significant difference with respect to sex ratio, WBC count, tumoral syndrome, and immunophenotype between *TEL-AML 1* positive and negative BCP-ALL. For MRD studies, total RNA, extracted at diagnosis from *TEL-AML1* positive patients, was serially diluted. The RT-PCR-JSO assay allowed us to detect *TEL-AML 1* transcripts up to a dilution of 10^{-5} in all cases. Remission samples of the 16 pts were serially cryopreserved. 8 / 9 analysed pts are evaluable at the end of induction therapy. Four expressed detectable transcripts but at a low level ($<10^{-3}$), two of them becoming negative subsequently. In 4 cases the amount of *TEL-AML1* transcripts fell below the detectability threshold at D40 and remained negative in subsequent analyses. Only one pt had three successive positive results at a dilution of 10^{-5} over a 8 month follow-up. None of these 16 pts has relapsed with a median follow up of 15 months (1-31). **Conclusions**: 1) a cryptic t(12;21) can be demonstrated in one fourth of the children affected with a BCP-ALL and is not associated with high-risk features 2) RT-PCR-JSO assay is a sensitive tool for detection of MRD 3) A multicenter prospective study is currently underway in our group to try to clarify the clinical relevance of *TEL-AML 1* transcripts detection.

O-97

IMMUNOGLOBULIN (Ig) AND T-CELL RECEPTOR (TCR) GENE REARRANGEMENTS IN CHILDHOOD ALL

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Early and late toxicities of childhood ALL treatment have become a significant burden, and new strategies are needed to identify patients curable with less intensive therapy. ALL is a clonal B-/T-lineage disease. Similar to their normal counterparts, leukemic blasts have rearranged Ig and TCR genes. The unique nucleotide sequences of these are valuable for the diagnosis of ALL and for follow-up of minimal residual disease (MRD). In an ongoing Nordic multicenter study, Ig-heavy (IgH) and TCR-γ/δ rearrangements are analysed at diagnosis and MRD is quantified at days 14 and 28. PCR-analyses at diagnosis are performed on the junctional regions of the IgH, TCRγ, and TCRδ (the incomplete Vδ2-Dδ3 rearrangements) genes. Clonal rearrangements were detected in 71 of 72 patients (8 T-lineage) entered by 2/96. The last patient had buphenotypic feaatures. 54 patients had IgH (0 T-lineage), 37 patients TCRδ (0 T-lineage), and 30 patients TCRγ (x T-lineage) rearrangements. 38 patients had 2 and 6 patients 3 clonal markers. All non-ALL patients studied were PCR-negative. Since most methods for MRD-analyses are at best semi-quantitative, a new, competitive, semi-nested, clone-specific and reproducible PCR-technique was developed (sensitivity limit $1:10^5$). A significant number of patients had less than 1 malignant cell:10^3-10^4 normal nucleated BM-cells by d14. Patients who relapsed had the highest d28 MRD-level (all being in morphologic remission) ($p<0.05$). MRD by d14/d28 should be tested for stratification of treatment intensity following induction therapy.

O-98

RADIATION THERAPY FOR RHABDOMYOSARCOMA: Local Failure Risk for Clinical Group III Patients on IRS-II

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Purpose: A subset of pediatric patients with rhabdomyosarcoma were selected from all eligible IRS-II patients to assess the local and regional failure rates following radiotherapy and to determine patient, tumor, and treatment factors contributing to risk for local and regional failure.

Methods and Materials: The study population was selected from 532 eligible IRS-II Clinical Group III patients enrolled between 1978 and 1984. Excluded patients were: those with "special pelvic" primary sites whose protocol management restricted radiotherapy (n=123); and those who were off-study before the time protocol radiotherapy was indicated or in whom radiotherapy was omitted (n=47). A binary recursive partitioning model was used to identify subgroups of the remaining 362 patients at differential risk of local or regional failure.

Results: The 5-year, actuarial risk of local (only) failure was 17%, and the local (all) failure rate was 20%. Fewer than 10% of local failures occurred after 3 years. The risk of regional (nodal) failure was between 2% and 23%. By univariate analysis, factors not influencing local failure risk were: age, race, gender, adenopathy, and histology. Compliance with radiation treatment guidelines approached but did not achieve statistical significance as a predictive factor. By protocol, radiation dose was keyed to tumor size, and no correlation of dose with failure risk was observed; however, increasing tumor size did predict an elevated local failure risk. Primary tumor sites above the clavicle had a reduced risk of local failure. The binary recursive partitioning model identified a subset at high local failure risk: patients with primary sites in chest, pelvic region, extremity, or trunk; or tumor size >10 cm. Their local failure rate is 35% (compared to 15% for the remaining patients). The high-risk subset for regional (nodal) failure was composed of patients with initial node involvement and a primary site other than orbit, parameningeal, or trunk.

Conclusions: Radiation therapy and chemotherapy administered in accordance with (but not necessarily in compliance with) the Clinical Group III guidelines for IRS-II achieves sustained local control in the majority of patients. Children with primary tumors of unfavorable site or maximum diameter >10 cm warrant more aggressive treatment to reduce their local failure risk.

O-99

RADIATION-THERAPY (RT) IN THE INITIAL MANAGEMENT OF LOCALIZED MALIGNANT MESENCHYMAL TUMORS (MMT). UPDATE OF THE SIOP MMT 89 STUDY

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Rationale. Based on the promising results of the previous MMT 84 study, in which RT was omitted in favorable cases, RT was restricted in the subsequent MMT 89 study to high risk patients, i.e. parameningeal sites and residual disease following an initial course of chemotherapy (IVA regimen : Ifosfamide, Vincristine, Actinomycin) ± surgical resection of the primary. Total median dose was 45 Gy ± boost, administered either with a conventional fractionation (QD : 5 daily sessions of 1.8 to 2 Gy) or with a hyperfractionated accelerated (HART) regimen (BID : 10 daily sessions of 1.25 Gy to 1.5 Gy according to normal tissue tolerance). The BID fractionation was optional and expected to provide improved local control without increased long term toxicity. Target volume encompassed the initial tumor volume in parameningeal sites (PM) and the residual in other sites.

Patients. From 1.1989 to 11.1995, 280/784 (36 %) children and adolescents below the age of 16 years received RT. 139 (50 %) presented with a PM site. 220 (79 %) were treated QD, 41 (15 %) BID and 17 (6 %) with brachytherapy (BT).

Tolerance. Acute tolerance was significally better in the QD group compared with the BID one, both for cutaneous (p.005) and mucosal (p.001) reactions, although treatment interruptions were not far different (6 and 9.5 days respectively).

Outcome. Adjusted for sites, DFS at 3 years differed significantly for QD (64 %) and BID (45 %) (p.02) but S (68 vs 61 %) and local failures FS (64 vs 54 %) didn't. No clear dose-effect relationship was evidenced on local control either.

Conclusion. In MMT 89, RT although directed to unfavorable cases, provided good survival (3 years : 70 % including BT, vs 75 % for the entire population). The HART regimen failed to show any benefit on local control and survival at the price of an increased acute toxicity.

O-100

CONTIGUOUS CENTRAL NERVOUS SYSTEM (CNS) RELAPSE IN PATIENTS WITH NON-METASTATIC CRANIAL PARAMEMINGEAL RHABDOMYOSARCOMA (CPM/RMS): A REPORT FROM INTERGROUP RHABDOMYOSARCOMA STUDY-III, -IV PILOT, AND -IV.

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424 previously untreated patients (pts) <21 years of age with localized CPM/RMS were entered on 3 sequential IRS trials from 1984 to 1995. Of them, 356 (84%) achieved a complete or good partial response (≥ 50% shrinkage) after multiagent chemotherapy and radiotherapy (RT). High-risk pts with cranial nerve palsy, cranial base bony erosion, and/or intracranial extension (ICE) of tumor were given RT as soon as possible after diagnosis; RT for low-risk pts began at week 6 to 9. In IRS-III (1984-87), all high-risk pts received whole-brain RT at 24 to 30 Gy; in IRS-IV Pilot (1987-91) only pts with ICE received whole-brain RT, and in IRS-IV (1991-ongoing) whole-brain RT was withheld. High-risk pts also received intrathecal chemotherapy for 18 months in IRS-III and for 5 months in IRS-IV Pilot, but none in IRS-IV. Overall, 20 of the 356 pts (5.6%) developed increasing CNS tumor growth at a median of 52 weeks after diagnosis; only 1 pt survives. 17 of the 20 pts were high-risk at diagnosis. CNS relapse rates were 4.7%-IRS-III, 3.6%-IRS-IV Pilot, and *8.5%-IRS-IV*. The types of CNS relapse were meningeal seeding (N=13, including 9 with tumor cells in CSF) and localized CNS tumor enlargement without meningeal spread (N=7). Preliminary RT data review showed that 8 pts were treated per protocol regarding dose, volume, and start date; 1 other received a relatively high dose, and 2 are yet unreviewed. The remaining 9 pts received a low dose (N=4) or limited volume (N=3), or started > 6 weeks late (N=2). Careful attention to RT dose/volume/timing requirements in pts with high-risk CPM/RMS should maximize disease control without the need for whole-brain RT and intrathecal chemotherapy. Supported in part by USPHS Grant CA-24507.

O-101

IS IT POSSIBLE TO CURE BLADDER / PROSTATE RHABDOMYOSARCOMAS (RMS) WITH CONSERVATIVE SURGERY? THE EXPERIENCE OF THE INTERNATIONAL SOCIETY OF PEDIATRIC ONCOLOGY.

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58 children with primary non metastatic (stage I-III) RMS of the bladder and/or prostate were enrolled in the MMT84 and MMT89 studies from 1.4.84 to 30.6.93. All received initial chemotherapy (CT). Local treatment was decided according to the response to initial CT: only patients (pts) who failed to achieve complete remission with chemotherapy were given local therapy.

The median follow-up time of survivors is 62 months (24 - 113 m).

A surgical pocedure was performed in 37 patients (pts) with residual mass after initial CT; 13 pts underwent total cystectomy (TC) and **24 (19 boys and 5 girls) underwent conservative surgery** (CS), e.g. without urinary diversion. The procedure was partial cystectomy in 13 pts, PC + total prostatectomy (PT) in 1 pt, PC + partial prostatectomy (PP) in 3 pts, TP in 1 pt, PP in 5 pts and tumorectomy in 1 pt. Nine pts received complementary radiotherapy (RT): external radiotherapy (ER) (2 pts), brachytherapy (BT) (6 pts), and ER + BT (1pt).

Outcome: two pts developed metastases after PC and died. One pt presented with an ovarian regional relapse after PC, was cured by ovariectomy. Eight pts relapsed locally (3 after PC, 4 after PP and 1 after tumorectomy). They were treated by repeated CS + ER or BT (2); TC (2); BT (2); one refused treatment, one died from toxicity of CT. Among these 8 pts, 3 are disease-free with 19, 36 and 98 months FU. **Among the whole group of 24 pts with CS, 17 (71 %) are alive,** 13 in first remission and 4 after treatment of relapse (one after TC).

In conclusion, CS is an alternative to total cystectomy for bladder/prostate RMS when tumor site makes it technically feasible. It should be considered before total cystectomy in children with a residual tumor after initial CT. CS avoids urinary diversion but conveys a risk of incontinence. Bladder function is being studied in this group of patients.

O-102

DEVELOPMENT OF A "RESECTABILITY SCORE" ACCORDING SITE, T-STATUS, AND TUMOUR SIZE FOR PREOPERATIVE ASSESSMENT OF RESECTABILITY IN SOFT TISSUE SARCOMA. A RETROSPECTIVE ANALYSIS.

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Decision basis for the preoperative question "Is a tumour R0 resectable or not?" is influenced by several conditions: Kind and quality of preoperative imaging, presumed histological diagnosis, experience of the first assessing centre and sometimes even specialisation and "courage" of the attending surgeon. Out of our historical CWS data we elaborated parameters for - mediately - predicting achievable resection status:

Exact site: Distinct sublocalisations were characterised, that were never or often R0 resectable in retrospective analysis. This sites were defined as "High-Risk-Regions" (5 pts., for example trigonum vesicae, parameningeal, orbit, shoulder with bony arrosion) or "Low-Risk-Regions" (1 pt., for example forearm without bony arrosion, cheek, paratesticular).

Tumour size: Tumour size was categorised according the max. diameter in tumours below 3 cm (1 pt.), 3 to 5 cm (2 pts.), 6 to 10 cm (4 pts.) and more than 10 cm (5 pts.).

T-status: As an indirect measure for the invasiveness the T-status (limitation of the tumour to tissue of origin or not) according MRI/CT was categorised in T1 (1 pt.), TX (3 pts.), and T2 (5 pts.)

Despite the fact, that these three parameters could not be handled independently from each other the sum of the pts. of each parameter gives a quite close hint on the achievable resection status and should furtheron be named as "resectability score". A score of 3 pts. for example (i.e. 2 cm T1-tumour of the forearm (1+1+1=3)) led in retrospective evaluation of 1008 mostly juvenile pts. with chemotherapysensitive soft tissue sarcoma (RMS, EOES/PNET, SS) of the CWS-studies 81, 86, and 91 in only 4% of all cases to *a gross residual tumour (pT3b) after initial surgery, while tumours with a score of 14* pts. for example (i.e. 7 cm T2-tumour of the shoulder (5+5+4=14)) were only in 1% of all cases complete resectable (pT1/2) and in 92% a gross residual tumour (pT3b) had to be left after initial surgery.

In summary this developed resectability score is in no way able to replace critical preoperative assessment of the surgeon in order to predict resectability. Even in tumours with a very high resectability score R0 resection may be achievable by the surgeons competence. Nevertheless the score could give the non-operative disciplines (oncologists, radiotherapists, etc.) an argument for preoperative discussion of resection trials and could encourage inexperienced surgeons to transfer patients with difficult tumours to specialised centres. A very important side-effect of this preoperative considerations could be the establishment of a regular preoperative assessment even in cases with only clinical suspicion of a malignant process. The validity of the described resectability score however should receive further investigation in a prospective way within the actual CWS-96 protocol. (supported by BMFG and Deutsche Krebshilfe).

O-103

Abstract withdrawn.

O-104

EXTENDED PRETHERAPEUTIC STAGING BY TOTAL-BONE MRI AND PCR ANALYSIS OF BONE MARROW IN PATIENTS WITH EWING TUMORS

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Most relapses in patients with Ewing tumors (ET) are caused by systemic failure, suggesting that dissemination has taken place in most patients long before surgery of the primary tumor. Staging for metastatic bone/bone marrow disease is routinely performed by radioactive bone scan and bone marrow aspirations investigated by light microscopy. In order to increase sensitivity for the detection of metastatic disease, total-bone MRI (TB-MRI) and bone marrow RT-PCR investigation for ET specific gene rearrangements were included in the pretherapeutic evaluation of 15 patients with ET (7 localized, 8 metastatic disease according to standard criteria). In none of the 7 patients with localized ET additional bone or bone marrow metastases were detected by TB-MRI. However, 2/7 cases were RT-PCR positive in the bone marrow. In 3 out of 4 patients with bone metastases, additional bone lesions were detected by TB-MRI. TB-MRI revealed no bone lesions in the 4 patients with isolated lung metastases while RT-PCR positivity was revealed in 3 of these cases and in all 4 patients with bone metastases. Our preliminary results underline that TB-MRI is able to reveal additional lesions in patients with bone metastases. However, RT-PCR of bone marrow increases the sensitivity in the detection of tumor dissemination in ET patients with metastatic and with so-called "localized disease". The clinical impact of this extended pretherapeutic staging program has to be established in a larger number of cases.

O-105

SURVIVAL IN PRIMARY DISSEMINATED OR RELAPSED EWING'S TUMOR - EICESS/CESS DATA

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Background: In the Cooperative Ewing's Sarcoma Studies of the German Society of Pediatric Oncology and Hematology since 1981, combination chemotherapy was used with surgery and/or radiotherapy. Primary and secondary disseminated patients (pts) were treated in high risk protocols. 14-18 GY whole lung irradiation was administered for pulmonary involvement. Some pts received myeloablative megatherapy followed by bone marrow or peripheral stem cell rescue.

Methods: Overall survival (OAS) rates were estimated by Kaplan-Meier analysis. Prognostic factors were identified by Cox and logrank analysis.

Results: Of 134 primary disseminated pts registered from January 1990 to December 1994, 54 pts have died until July 1995 (median time of observation 34 months). 108/134 primary metastatic pts were evaluable for lifetable analysis. Estimated OAS four years after diagnosis was 0.39 for these compared to 0.58 for all 385 study pts. Isolated primary lung or bone/BM metastases fared better (0.50 resp. 0.41) than combined lung/bone/BM metastases (0.15), $p < 0.001$. Megatherapy did not influence outcome. In 53 pts with primary pulmonary involvement, whole lung irradiation improved outcome (0.53 vs. 0.13, $p = 0.0001$).

104 of 272 pts without primary metastases registered from 1981 to 1990 relapsed, 89 pts have died until July 1995 (median time of observation 7 years after relapse). Estimated OAS was 0.10 ten years after relapse. A disease-free interval from initial diagnosis to relapse exceeding two years increased prognosis to 0.33 from 0.00 ($p < 0.001$).

Conclusion: Survival in primary disseminated Ewing's sarcoma is poor with a Kaplan-Meier probability of 0.39 four years after diagnosis. Whole lung irradiation improves prognosis in pts with pulmonary involvement (0.53). 10 years after relapse of primary non-metastatic Ewing's tumor prognosis is even worse (0.10), except for late relapses (0.33).

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O-106

THE ROLE OF MEGATHERAPY (MGT) FOLLOWED BY STEM CELL RESCUE (SCR) IN HIGH RISK EWING TUMORS (ET). 11 YEARS SINGLE CENTER EXPERIENCE.

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Aim:To eventually define potential risk-groups in this cohort of poor prognosis ET patients.

Patients and methods: Between July 84 and January 96, 27 ET patients (14 male, 13 female) received treatment intensification by MGT/SCR in our institution. The median age at diagnosis (dx) was 12,35 years (range, 3.5 to 25.5 years). Primary tumor site was pelvic in 10/27 pts, ribs in 7/27pts and extremities in 10/27pts. Thus 17 pts had central tumors at dx .Histology at the time of dx was described as follows: 11pts were assigned to the PNET group and 16 pts were diagnosed as Ewing sarcoma. Conventional first line chemotherapy (cCHT) was given according to the consecutive (EI)CESS studies. All patients submitted to MGT/SCT either had primary metastatic disease (18 pts) or had relapsed (9 pts). All relapse pts had responsive disease prior to the procedure. The median observation time is 56 months (range, 2 months to 140 months). Stem cell source was autologous bone marrow (BM) in 6 pts, peripheral stem cells in 13 pts, both of the latter two in 2 pts and finally 6 pts received allogeneic BM. The MGT regimen included melphalan, VP16 +/- CBDCA (ME(C)) and involved total body irradiation until 1994 in 20 pts whereas more recently irradiation was given to involved sites only.

Results:The overall survival was poor in this patient cohort with a 23% survival rate at the median observation time. Survival rate for pts in first or second CR was 26% and for pts in first PR or sensitive relapse 21%. However, when patients were divided by the given diagnosis, Ewing sarcoma pts had a more favourable prognosis of 40%, whereas PNET pts had a very poor outcome of only 7%. Allogenic BMT improved outcome demonstrated by a 50% survival rate. However, results have to be regarded with caution since pts numbers are small. None of the latter 2 results reached statistical significance.

Conclusion: Overall results are still poor in metastatic Ewing tumor pts and the TBI/ME(C) regimen has only gained about 10% in survival rates in our hands in comparison with historical data. A reviewed histology will be presented.

O-107

END-INTENSIFICATION WITH HIGH-DOSE CHEMOTHERAPY, TOTAL BODY IRRADIATION, AND AUTOLOGOUS BONE MARROW TRANSPLANT FOR HIGH-RISK LOCALIZED EWING'S SARCOMA

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Since 1982, patients with localized Ewing's sarcoma of bone have been stratified by tumor size, and patients with large primary lesions (>8 cm in maximum diameter) have been treated more intensively in an attempt to improve survival. Patients with small primary lesions have received local therapy (radiation or surgery) and 6-8 cycles of vincristine, doxorubicin, and cyclophosphamide (Protocols SR-1, SR-2). Patients with large primary lesions have received 6 cycles of standard chemotherapy followed by end-intensification with either vincristine, doxorubicin, and cyclophosphamide and total body irradiation (HR-3) or etoposide, cyclophosphamide, and total body irradiation (HR-4) followed by autologous bone marrow transplant. From 1985 to 1993, 16 patients <21 years of age were entered on the standard-risk protocols (SR-1, SR-2) and 16 on the high-risk protocols (HR-3, HR-4). All 16 patients on HR-3 and HR-4 had pelvic, trunk, or proximal extremity primary tumors. Of the 16 patients, 1 refused transplant (died after distant relapse); 2 died of transplant-related toxicity; 3 relapsed; and 10 are alive without evidence of recurrence. Five-year survival and event-free survival rates were 75% and 62%, respectively; for patients treated on high-risk protocols; both 5-year rates were 55% for patients on standard-risk protocols. Survival rates appear superior for patients treated on the high-risk protocols than for historical controls at the University of Florida.

O-108

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) IN HIGH RISK EWING' SARCOMA (HR-EwS)

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The prognosis of HR-EwS (large pelvic mass and/or presence of metastases at diagnosis) is still poor; in April '93 we started a multicentricstage II/III study with intensive chemotherapy and PBSC rescue in order to evaluate the feseability of such approach and to improve disease free survival.

MATERIAL AND METHODS: 43 patients (pts) were enrolled (23 adults/20 children; 28 males/15 females), 27 metastases and 16 pelvic mass. Treatment schedule consists of a high dose cytoreductive-mobilizing phase with 2 courses of HyperVAC (VCR 2 mg/sqm, CTX 2,2 mg/sqm, ADM 45 mg/sqm x 2 days), alterned to 2 courses of CE (VP-16 200 mg/sqm x 3 days, CTX 4 g/sqm), supporeted by G-CSF (250 mcg/sqm), in order to improve dose intensity and enhance the PBSC mobilization. This phase is followed by surgery and/or radiotherapy plus 2 cycle standard dose of VAC alterned to 2 cycle Ifosfamide/VP-16. Pts with VGPR or RC receive myeloablative regimen (Busulfan 4 mg/kg x 4 days, VP-16 600-800 mg/sqm x 3 days, Thiotepa 300 mg/sqm) plus PBSCT. Minimal Residual Disease (MRD) was evaluated by PCR method on PBSC collection in a subset of 4 pts.

RESULTS: as of February '96, 33 pts completed the cytoreductive phase and are evaluable for disease status. Haematologic toxicity was grade 3-4 in all induction cycles and in 80% of consolidation cycles, without organ toxicity and toxic death during PBSCT. Local treatment was surgery in 10 pts (8 adults), radiotherapy in 18 adults and 15 children. Out of 18 pts, who are elegible for transplant, 2 refused procedure and 16 (9 young adult, 7 pediatrics) underwent to PBSCT. Sixtheen pts received a median (min-max) number of 4,78 (2,63-9,07) x 10^8/kg MNC, 144,5 (33-369) x 10^4 /kg CFU-GM and 14,6 (2,48-33,2) x 10^6/kg CD34+. After a median follow-up of 16 months (range 6-30), 21 pts are alive with NED, 8 pts relapsed (5 died), 3 had progression disease (all died), 1 is alive with disease. Overall survival and the event free survival respectivily is 53,2% and 41%.

CONCLUSIONS: The regimen is feasable and has efficacy both as PBSC mobilization and cytoreductive action on primary mass and on metastases. A longer follow-up and a higher number of transplanted pts are needed to evaluate long term efficacy of this innovative approach.

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O-109

TREATMENT OF METASTATIC EWING'S SARCOMAS (ES) WITH BUSULFAN AND MELPHALAN CONSOLIDATON HIGH DOSE CHEMOTHERAPY (HDCT). A STUDY OF THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP)

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In the EW88 study of the SFOP, 25 patients (pts) with metastatic ES were treated with conventional chemotherapy (CT) from 1.1988 to 12.1990. The 3 year DFS and overall survivals were respectively 12 % and 25 % (MPO 21:572, 1993). The efficacy of HD busulfan in relapsing patients prompted us to design a new study for metastatic pts based on Busulfan and melphalan.

From Jan.1991 to Jan.1995, 44 pts entered the EW91 study. Sites of metastases were isolated lung (23), bone (6), combined (15). Bone marrow involvement was present in 8 pts. Median age was 12 years (1-24). Induction CT consisted of cyclo. 150 mg/m2 p.o. x 7 days followed by doxorubicin 35 mg/m2 IV on day 8 for 5 courses beginning days 1, 15, 29, 50 and 71, followed by 2 courses of Ifosfamide 1.8 g/m2+ VP16 100 mg/m2 for 5 days. Pts who achieved complete response (CR) or very good partial response (VGPR) of metastases were HDCT consisting in Busulfan (600 mg/m2) and melphalan (140 mg/m2) followed by autologous stem-cell transplantation. Local therapy was performed either before or after HDCT.

9 / 44 progressed under initial CT; 12 and 22 achieved VGPR or CR of metastases and underwent HD CT; 2 died from toxicity related to the procedure, 13 relapsed after. 20 are alive in continuous remission with a median follow-up from HDCT ranging from 3 to 50 months from diagnosis (median 30 months). The 3 year disease-free survival of the whole cohort was 41 % and the overall survival was 62 % for the whole cohort. It was respectively 52 % and 76 % for the selected group of 34 patients who underwent HDCT. Response to initial conventional CT was the only prgnostic factor.

Despite the need of longer follow-up, this compares favorably with results observed after treatment with conventional CT alone.

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O-110

SEQUENTIAL MOBILISATION OF PERIPHERAL BLOOD PROGENITOR CELLS IN PATIENTS WITH BONE TUMOURS: IMPLICATIONS FOR HAEMOPOIETIC SUPPORT OF INTENSIVE THERAPY

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Adjuvant chemotherapy has improved survival for children and young adults with osteogenic sarcoma and Ewings' tumour of bone (cisplatin/adriamycin and ifosfamide based chemotherapy respectively). Myelotoxicity is the dose limiting toxicity for both treatment regimes and although growth factors currently used can reduce neutropaenia they have no consistent effect on platelet recovery. Peripheral blood progenitor cells (PBPC) can, however, hasten recovery of both neutrophils and platelets following intensified chemotherapy. With a view to using mobilised PBPC's to support chemotherapy, patients with newly diagnosed osteogenic sarcoma, Ewings' tumour or rhabdomyosarcoma were treated with G-CSF (5 mcg/kg) at day 5 post chemotherapy until neutrophil recovery during sequential chemotherapy (4 courses). Blood samples were obtained on alternate days and the white cell count, CD34 + analysis and granulocyte-macrophage colony forming cell assays (GM-CFC) were performed. The median peak day of progenitor release was day 15 in group 1 (osteogenic sarcoma group, n=5) and day 11 in group 2 (ifosfamide based chemotherapy group, n=7). The mean GM-CFC/ml blood was 439 in group 1 and 777 in group 2. PBPC mobilisation during the first cycle was compared with cycle 4: in group 1, mobilisation was extremely poor by course 4, however the patients in group 2 demonstrated less reduction in the progenitors. Similar results were obtained when earlier progenitors were assayed using 2-stage long-term bone marrow culture. The results suggest that ifosfamide based chemotherapy in conjunction with G-CSF is effective in mobilising PBPC's and this is sustained through 4 courses. In contrast cisplatin/adriamycin with G-CSF is less effective during the first course and by course 4 mobilisation is extremely poor suggesting that early collection of PBPC in patients with osteogenic sarcoma is necessary for optimal haemopoietic support.

O-111

PHASE II STUDY OF HIGH-DOSE THIOTEPA (HDT) AND HEMATOPOIETIC STEM CELL TRANSPLANTATION (SCT) SUPPORT IN CHILDREN WITH METASTATIC OSTEOSARCOMA

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Between 1992 and 1996, 11 children with metastatic osteosarcoma entered a phase II study of HDT (900 mg/m²) with autologous SCT support.Their median age at the time of the HDT was 14 years (8 - 19 y). Three children had metastatic disease at diagnosis, 8 had a localized tumor and experienced metastases thereafter. For the previous tumoral episodes, all had been treated with several conventional chemotherapy regimens with a median number of 6 drugs (4-8). Surgical excision of the primary tumor had been performed in all, surgical excision of metastases had been performed in 8 pts. At time of HDT, all had measurable disease, at metastatic sites. One and 2 patients were in 1st and 2nd PR respectively, and 3, 3 and 2 were in 2nd, 3rd and 5th disease progression. **Results :** Toxicity was mainly marked by hematological and digestive side effects. Neutropenia $< 0.5 \times 10^9$/l and thrombocytopenia $< 50 \times 10^9$/l lasted for a median duration of 9.5 d (7-29) and 32 d (7-377) respectively. Digestive toxicity consisted of grade $\geq$ II vomiting in 7 pts, grade $\geq$ II diarrhea in 5 pts. Hepatic toxicity consisted of reversible biologic disorders in 3 pts and a mild hepatic veno occlusive disease in 1. **Response** was assessed by both imaging and histological evaluation, post SCT surgical excision of the residual metastases being performed in 8/11 pts. Following HDT, 1 CR, 7 PR, 3 NR were observed (response rate 73 %). **Survival :** event free survival at 24 months post SCT was 31 %. Three patients are alive in first CR post SCT (6m +, 35m +, 36m +), 1 is alive in 2nd CR post SCT (14m +) 1 is alive with stable disease (5m +), 4 died of disease progression (6,10,17,19 m post SCT), treatment of residual disease after HDT is in progress for 2 patients. **Conclusion :** This first phase II study of high-dose alkylating agent in poor risk osteosarcoma demonstrates the efficacy of this approach in this disease. The role of HDT in the treatment strategy of poor risk osteosarcoma should be discussed in the future protocols of high-risk patients.

O-112

ANALYSES OF THE MANCHESTER CHILDREN'S TUMOUR REGISTRY (MCTR) CHILDHOOD LEUKAEMIA INCIDENCE DATA USING SPACE-TIME CLUSTERING RELATED METHODS.

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A body of evidence is accumulating in support of the role of infections in childhood leukaemia. Variations in incidence in time and space might therefore be expected.The MCTR represents an ideal data set in which to investigate time-space interactions having a consistently high level of ascertainment and diagnostic accuracy over a long period.Using these data,space-time(Knox)tests were performed with(i)the single a priori definition of "close in space" as less than 5km and "close in time" as less than 1 year:(ii)geographical distance replaced by "distance to N^{th}"nearest neighbour with N chosen so that the mean distance was 5km, thereby adjusting for the effects of different population density on the Knox test. Highly significant evidence of time-space clustering was found.This was particularly evident for all leukaemias aged 0-4;ALL aged 0-4;ALL aged 2-4. Comparison analyses of other age and diagnostic groups failed to detect significant departures from the random null situation. Clustering was found in relation to place and time of diagnosis but not place and time of birth.There was strong evidence of clustering in relation to place of birth and time of diagnosis.Locations at birth and diagnosis were classified as urban,rural or mixed and case-children classified according to whether they had moved from less to more dense or more to less dense population areas between birth and diagnosis.Significantly more children with ALL diagnosed during the childhood peak had moved from less to more dense population areas than from more to less.These results would support a hypothesis relating to location at some time before diagnosis and are consistent with an aetiological role for infections.

O-113

EFFECTS OF MASS SCREENING ON AGE-SPECIFIC INCIDENCE OF NEUROBLASTOMA

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Using data from Japan Children's Cancer Registry, the age-specific incidences (per 1,000,000 population) of neuroblastoma were estimated. Before the neuroblastoma screening program started, the age-standardized incidence rates of neuroblastoma ranged from 7.5 to 9.0 for children younger than 15 years old. After introduction of screening program, the incidence rate rose to 19.2. Incidence rate of neuroblastoma in children younger than 1 year between 1989 and 1992 was 146.5 whereas the incidence rates were from 23.6 to 33.7 for the 3 5-year periods before screening. This increase in incidence for infants seemed to be accompanied by a corresponding decrease in those for children of 2 and 3 years of age. That is, ratios of incidence rates of 1989-1992 (post-screening) to 1979-1983 (pre-screening) for children of 2 and 3 years of age were 0.91 (95% confidence interval: 0.61-1.35) and 0.83 (95% confidence interval: 0.56-1.26), respectively. However, this decrease was not significant and could not fully explain a large increase in incidence in infants. This discrepancy was thought to be due to detection of otherwise spontaneously regressing tumors. We suggest that current screening program should be discontinued until non-invasive methods of discriminating tumors with poor prognosis from spontaneously regressing tumors become available.

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O-114

VARYING INCIDENCE OF NEUROBLASTOMA IN EUROPE: HOW EFFICIENT IS DIAGNOSIS?

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Age-standardised incidence rates for neuroblastoma were derived using data from 4 European countries. These were France (population under 15 years, 11.6M), Germany (8.9M), Austria (1.3M) and Britain (10.3M).

Between 1987 and 1991, 1652 cases of neuroblastoma were registered in these 4 countries (France 625: Germany 470: Britain 488: Austria 69). The age-standardised incidence of neuroblastoma in France was 12.5 cases/ million/ year (95% c.i. 11.5 - 13.5). This was significantly higher than the incidence in Germany (10.9, 9.9 - 11.9) and Britain (10.1, 9.2 - 11.0), and similar to the value in Austria (11.7, 9.0 - 14.5).

Disease stage (Evans) was known in over 95% of cases. In Britain, the incidence of low stage (I, II, III) and IVS disease was significantly lower than in other countries, while the incidence of stage IV disease was higher.

Poisson regression analysis was used to examine underlying patterns in the incidence rates. This showed that the 'deficit' of low stage disease in British children predominantly affected children under 1 year of age while the 'excess' of stage IV disease was most pronounced in children aged between 1 and 2 years. These findings, along with observed differences in survival rates in these European countries, suggest that in Britain there may be a failure to detect early stage disease.

In Germany, 34% of the cases were detected at routine health examinations or investigations into an unrelated condition, compared to 8% in Britain. The likelihood of low stage disease being diagnosed may depend on the health-care system of the country, and underdiagnosis of these cases may be occurring in Britain. This finding has important implications for the evaluation of neuroblastoma screening programs.

O-115

GERMLINE TP53 MUTATIONS AMONG 38 FAMILIES WITH LI-FRAUMENI (LF) SYNDROME.

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We previously reported 6 germline TP53 mutations in 12 families conforming to syndrome criteria of Li etal Cancer Res.48,5358,1988(LFS) and 1 mutation in 9 families with features of the syndrome but applying less restrictive criteria(LF-like).To further clarify the role of TP53 analyses of 17 additional families are in progress.For each proband constitutional DNA was amplified by PCR and exons 1-11 including splice junctions,were analysed by direct sequencing.Where a mutation was detected,other family members were tested and loss of heterozygosity(LOH)in tumour tissue from multiple members was analysed. So far germline TP53 mutations have been found in 6 LFS and 3 LF-like families. 5 involve known mutational hotspots but 4 are novel mutations including a large deletion in exon 1/ intron 1 and a missense mutation in exon 10. Combining all our results in 32 families in whom analyses have been completed,11/19(58%)LFS families and 4/13(31%)LF-like families carry germline TP53 mutations.Mutation positive families are characterised by adrenocortical carcinoma, high frequency of CNS tumours and low frequency of leukaemia/lymphoma.Patterns of LOH varied within families and between tumours;loss of wild-type,retention of both mutant and wild-type and loss of mutant alleles being observed.In two nuclear families where mutations occurred in successive generations in cancer-affected individuals, other members with typical LFS cancers did not carry the mutations.We conclude(1)analysis of conserved exons only is inadequate(2)there are phenotypic differences between mutation positive and mutation negative LF families(3)the relationship between germline TP53 mutations and cancer risk in LF families is more complex than originally thought

O-116

FERTILITY AFTER TREATMENT FOR LEUKEMIA DURING CHILDHOOD

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Improved survival after treatment for childhood malignancies has brought new concerns to families and patients. Among them is the possibility of loss of fertility, either immediate or delayed. Fertility in leukemia survivors is a major issue that has not been satisfactorily addressed. We studied fertility (defined as rates of first pregnancy) in a large cohort of long-term survivors of childhood leukemia and their sibling controls. Study eligibility criteria were: treated before 1990 on Childrens Cancer Group protocols; diagnosis before age 21, and at least 18 at follow-up. Subjects were interviewed by telephone between 1990 and 1992. 448 survivors and 361 sibling controls reported ever having sexual intercourse and were thus eligible for this analysis. Age at first pregnancy was similar for both groups (19 years), but controls were older at interview (26 vs 23) and more likely to have been pregnant, or fathered a pregnancy (48% vs 32%).

To control for age differences we did a person-years analysis looking at rates of first pregnancy at two age intervals. Overall, both male and females survivors had lower rates of first pregnancy than controls during ages 18-21 (RR=0.80 and 0.61, $p<.05$, respectively) but first pregnancy rates did not differ from controls at ages over 21 (RR=1.01 and 1.19 respectively). Male, but not female, survivors treated with 2400 cGy cranial and craniospinal radiotherapy had significantly lower fertility than males receiving no radiation therapy (RT) at all ages of follow-up ($p<.05$). Planned analyses will evaluate effects of cranial vs spinal RT.

These results are consistent with postponement of fertility on the part of leukemia survivors but also indicate the possibility of lasting gonadal damage in some survivors treated on older protocols.

O-117

HEALTH-RELATED QUALITY OF LIFE DURING POST-INDUCTION CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN REMISSION

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Using a multi-attribute approach, we and others have reported on the comprehensive health status of survivors of cancer in childhood, after completion of therapy. However, there is a dearth of information about health status and associated health-related quality of life (HRQL) in children and adults with cancer, during the treatment process; a deficit which this study was intended to redress. All children (n=18) receiving "maintenance" chemotherapy for acute lymphoblastic leukemia at a single institution were assessed prospectively through a single cycle of therapy. Patients, family members and health care professionals used the Health Utilities Index Mark 2 (HUI2) and Mark 3 (HUI3) classification systems, and detailed descriptions of temporary states of health, to provide independent assessments of health status at weekly intervals. Utility scores were derived for each comprehensive health state and for selected single attributes in the HUI2 system, and for the temporary health state descriptions. The classification of subjects into the most appropriate temporary health states was challenging even for older children and some of the parents. The HUI instruments were used much more easily and produced highly comparable information. The most frequently affected attributes were pain, emotion and mobility/ambulation; in that order. The global ($p=0.005$) and specific morbidity burdens were, as predicted, greatest in the middle of the cycle of chemotherapy, reflecting the toxicity of steroid use. HUI2 demonstrated moderate responsiveness with an intra-class correlation coefficient of 0.43. Significant changes in health status and HRQL were detected during the cyclical administration of therapy to children with ALL. The HUI are valid and responsive instruments for quantifying the burden of morbidity during the treatment of cancer in childhood. These are sensitive tools which can be used in a wide array of other circumstances to quantify changes in health status.

O-118

HEALTH STATUS OF LONG TERM SURVIVORS AFTER MYELOABLATIVE THERAPY AND BONE MARROW TRANSPLANTATION IN CHILDREN

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Aims. To define frequency and severity of late effects in a series of 85 children surviving more than 2 years after bone marrow transplantation.

Patients and methods. All children submitted to myeloablative therapy and bone marrow transplantation (BMT) and surviving free of relapse of disease more than 2 years after BMT underwent a physical, endocrinological, cardiac, polmonary, audiometric, orthopedic and ophtalmologic evaluation. The sequelae observed were scored 0-4 according to a grading system distinguishing defects in absent, asymptomatic, moderate symptomatic, severe, requiring major measures and life threatening.

Results. Thirty-four children received a conditioning regimen without TBI (13 undergoing an allogeneic BMT): 4 of them developed only endocrinal disfunctions. Fifty-one children received a TBI containing regimen (22 undergoing an allo BMT): 35 of them developed endocrinal disfunctions requiring substitutive therapy in 25; 2 pts had cardiological subclinical defects; 8 pts cases pulmonary subclinical defects; 12 pts, previously treated with cisplatin, had mild audiometric defects; 4 pts had head femoral osteonecrosis (1 requiring bilateral surgery) and 13 pts developed cataract (1 requiring surgery); 1 pt developed a fibrosarcoma which was radically excised and 1 pt suddenly died for unknown causes 3 years after BMT.

Conclusions. Long term surviving after BMT in childhood have an excess of endocrinological problems and major risk factors appear TBI, chronic GvHD and Busulfan.

O-119

RELATIVE OSTEOPENIA FOLLOWING TREATMENT FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA.

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Introduction: Osteopenia as a result of growth hormone deficiency (GHD) following cranial radiotherapy (CRT) is now recognised. Low dose CRT, 18-24 Gy is not often associated with frank GHD yet subtle changes in pulsatility have been described. The aim of this study was to compare bone mineralisation in long term survivors of childhood acute lymphoblastic leukaemia (ALL), treated with chemotherapy and low dose CRT, with both survivors of other malignancies (OM), who had received chemotherapy alone, and with healthy sibling controls.

Patients and methods: Bone mineral content (BMC, grams) and bone area (BA, cm^2) of the whole body (WB), lumbar spine (LS) and left hip were measured by dual energy X-ray absorptiometry in 34 (13 male) survivors of ALL (7.2-18.2 yrs), 20 (10 male) OM survivors (7.3-18.4 yrs), and 30 (16 male) healthy siblings (7.6-17.3 yrs). A predicted BMC was calculated using a regression formula derived from controls based on age, height, weight, gender, pubertal stage and BA (R^2 = 0.99, 0.94, 0.94 for WB, LS and hip respectively). The percentage of predicted BMC was expressed as a standard deviation score (SDS).

Results: Survivors of ALL and OM had completed treatment 6.4[2.6-12.8] and 6.7[1.5-11.8] mean [range] years respectively. Mean SDS [95% CI] at each site are given in the table. 33% of survivors of ALL had an SDS of less than -1.0 for all sites compared with 0% of the OM group and 7% of controls.

	WB*	LS†	Hip†
Sibs	0.0[-0.37 to 0.37]	0.0[-0.37 to 0.37]	0.0[-0.37 to 0.37]
ALL	-0.89[-1.43 to -0.35]§	-0.99[-1.33 to -0.65]#	-1.36[-1.83 to -0.89]#
OM	-0.56[-1.24 to 0.12]‡	-0.12[-0.68 to 0.44]‡	-0.63[-1.22 to -0.03]‡

(A.N.O.V.A. *p<0.05,†p≤ 0.0005.Post Hoc,#p<0.05 cf. sibs and OM,§p<0.05 cf. sibs,‡NS cf. sibs)

Conclusion: Children treated for ALL have a significantly reduced BMC at all sites when compared to healthy siblings and at the LS and hip compared to OM. These findings suggest that CRT (perhaps as a consequence of subtle abnormalities of GH release) and to a lesser extent chemotherapy (possibly as a result of corticosteroid therapy) predispose to long term osteopenia.

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O-120

CHROMOSOME 1P IN NEUROBLASTOMA: SUPPRESSORS AND PROGNOSIS

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Differences in tumor behaviour suggest that neuroblastoma can be divided into several clinico-biological entities.

We evaluated loss of chromosome 1p, 4p, 11q, and 14q, duplication of chromosome 17q, amplification of the N-myc oncogene and nuclear DNA content in a series of 89 neuroblastomas for their prognostic value. Loss of chromosome 1p appeared to be the most powerful prognostic factor of all genetic and clinical factors tested. It provides strong prognostic information when added to multivariate models containing the clinical prognostic factors age and stage, or ferritin and stage. Among stage I/II/IVs patients, the 3-yr. event free survival (EFS) was 100% for cases without 1p loss and 34% (SD 15%) in case of loss of chromosome 1p; among stage III/IV patients, 3-yr. EFS was 53% (SD 10%) and 0%, respectively.

Furthermore, chromosome 1p appears to harbour 2 different suppressor loci: a more proximal suppressor gene associated with N-myc amplification, and a distal suppressor, subject to genomic imprinting, inactivated in N-myc single copy cases. In the N-myc single copy tumours we show that the lost 1p36 alleles are of preferential maternal origin (16/17 cases) and that the commonly deleted region maps to 1p36.2-3. In contrast, all N-myc amplified neuroblastomas have larger 1p deletions, extending from the telomere to at least 1p35-36.1. These deletions are of random parental origin (18/30 maternal LOH).

In conclusion, we have evidence that chromosome 1p contains at least 2 different suppressor loci, loss of which identifies high risk neuroblastoma patients in all stages.

O-121

INDUCTION OF FAS ON B-CELL LEUKEMIAS BY TUMOR NECROSIS FACTOR-α AND INTERFERON-γ

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Only a minority of leukemias originated from progenitor B-cells express the Fas/APO-1 antigen on their surfaces. However, it has not yet been investigated if cytokines might be able to induce Fas/APO-1 on these leukemias and if subsequent activation of the Fas receptor could mediate apoptosis. Therefore, we investigated the expression of FAS/APO-1 and bcl-2, an inhibitor of the Fas death programm in three leukemic cell lines of different maturational stage by FACS analysis. FAS was not spontaneously expressed on any cell line. Stimulation of a common Pre-B-ALL line and a Pre-B-ALL line by IFN-γ, but not by TNF-α lead to a slight, but significant increase of Fas expression, whereas expression of bcl-2 was unchanged. Programmed cell death was not induced in IFN-γ treated cells by a cell death inducing Fas antibody. However, treatment of these cells by TNF-α alone was able to mediate significant apoptosis and subsequent cell death. In a leukemic B-cell line expressing surface Ig, TNF-α and to a less extend IFN-γ mediated a significant increase in Fas expression. Although bcl-2 was also upregulated upon stimulation with TNF-α, a cell death inducing Fas antibody could mediate apoptosis as well as cell death in TNF-α stimulated cells. In conclusion, depending on the maturational stage, different cytokines were able to induce Fas in B-cell type leukemias. However, upregulation of Fas was associated with inducibility of apoptosis and cell death only in a sIg+ B-cell leukemia. Thus, killing of B cell progenitor leukemias might rather be mediated by direct effects of certain cytokines than by induction of Fas-mediated programmed cell death. These results might help to establish immunotherapy protocols for treatment of B-cell derived leukemias.

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O-122

3T6/CD40-L STIMULATION OF B-CLL CELLS INCREASES THEIR IMMUNOGENICITY AND ENHANCES THE IN VITRO INDUCTION OF ALLOGENIC CYTOTOXIC ACTIVITY AGAINST THE ORIGINAL, UNMANIPULATED TUMOR

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The immunogenicity of B-CLL cells was investigated by in vitro primary and secondary mixed lymphocyte reactions (MLR) of autologous and allogeneic T cells against original and CD40-stimulated B-CLL tumor cells. B-CLL cells were stimulated during 22 hours of co-culture with CD40-L-transfected 3T6 fibroblast cells. Immunomagnetic separation techniques were used to isolate T cell subsets and B-CLL cells (>95% pure). Proliferation was determinded by measuring thymidine incorporation. Tumor-specific cytotoxicity was measured in a standard 4-hours chromium release assay. Primary MLR of autologous and allogeneic T cell subsets against original B-CLL cells did not result in proliferation or induction of tumor-specific cytotoxic T cells. In contrast, CD40-stimulated B-CLL cells strongly upregulated CD80, CD86 and CD54 molecules and elicited a strong proliferative response of allogeneic T cells. Moreover, allogeneic cytotoxic T cells were generated which were capable of lysing the original, unmanipulated tumor. This allogeneic response against CD40-stimulated B-CLL cells was partly inhibited by anti-CD80 or anti-CD86 antibodies and almost completely inhibited by CTLA-4Ig fusion protein, demonstrating the primary role of CD80 and CD86 costimulatory molecules. Secondary MLR of allogeneic T cells against original B-CLL cells, after primary MLR against CD40-stimulated B-CLL cells, again resulted in very low proliferation, suggesting that costimulatory molecules are required to maintain the response against a weak or even non-immunogenic tumor. We were not able to induce autologous tumor-specific cytotoxic T cells. Because the original B-CLL cells demonstrated no or only weak expression of CD80 and CD86 molecules, nonresponsiveness of autologous T cells against the CD40-stimulated B-CLL cells might have been the result from in vivo induction of tumor-specific anergy. However, in vitro primary allogeneic MLR against the original B-CLL cells did not result in anergy induction, when tested in a secondary MLR against CD40-stimulated B-CLL cells. In conclusion, although B-CLL cells potentially may carry tumor-specific antigens, they clearly lack the potential to stimulate T cells which eventually recognize them. Induction of CD80 and CD86 costimulatory molecules strongly enhances the immunogenicity of the B-CLL tumor cells in allogeneic MLR and these costimulatory molecules were clearly necessary to maintain the proliferative response. The failure to induce an autologous immune response may be the result of in vivo induction of anergy or deletion. ALternatively, inadequate presentation of putative tumor antigens in the context of MHC molecules may be the cause of nonresponosiveness.

O-123

INVOLVEMENT OF THE p53 AND p16 TUMOR SUPPRESSOR GENES IN THE DEVELOPMENT OF PEDIATRIC BONE TUMORS.

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Recent data suggest that deletion of p16 and the mutation of p53 tumor suppressor genes are common genetic events in the development of human cancer. As the molecular events leading to the development of pediatric bone tumors remains unclear, we investigated the possibility that a significant proportion of children with bone tumors harbor mutations of the p53 or deletions of the p16 tumor suppressor genes.

84 samples (26 DNAs from whole blood and 58 from fresh or paraffin-embedded tissues) from 58 pediatric patients with osteosarcoma (n=50) or Ewing sarcoma (n=8) were screened for the presence of p53 mutations using DGGE. Thirty-eight of the tissue samples (27 patients) were also available for p16 deletion analysis through coamplification of the exon 2 of the gene and a fragment of the T-cell receptor gene.

Eight out of the patients showed alterations of the p53 gene: four in exon 8, one in exons 5, 7 each and two in exon 6 (18,4% somatic mutation), one of which was a germline mutation (3,85%, a similar value to those previously reported, McIntyre *et al*, 1994; Toguchida *et al*, 1992).

We found that p16 is often homozygously deleted in pediatric bone tumors, 34,2% of the samples. The reason that deletion is so common in these tumors may be that it is an efficient mechanism for inactivation of this tumor suppressor gene.

As reported by Zhang *et al* (1994), we found that p16 is often deleted in tumors containing wild type p53 and is intact when p53 is mutated, suggesting that inactivation of one of these cell cycle regulatory elements may alleviate the need for alteration of the other.

We didn't find any correlation between presence of alterations in these genes and clinical parameters such as tumor grade or histologic subtype. However, all p16 deleted patients, except one, developed metastasis, but this series is still too short to draw stablish clinical correlations.

O-124

EVALUÁTION OF MINIMAL AND RESIDUAL DISEASE IN EWING TUMORS AT DIAGNOSIS AND DURING TREATMENT.

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The sensitive monitoring of the presence of tumors cells in blood and bone marrow provide with new perspectives in the management of patients with malignant disease. In this respect, Ewing's cells which express specific chimaeric transcripts as a result of the t(11;22)(q24;q12) chromosome translocation or of its variant t(21;22) constitutes a prototypic model since these transcripts can be detected by the highly sensitive reverse transcriptase-polymerase chain reaction technique. Using a nested PCR amplification procedure realized in a single tube, blood and/or bone marrow (BM) from 113 patients with Ewing's tumors were evaluated for the presence of tumor cells at various times before and during treatment. At diagnosis, 16/62 patients had circulating tumor cells. Although in some cases the spontaneous occurrence of these circulating cells could be established since the blood sample was evaluated before tumor biopsy, in other cases, the taking of blood was performed after the biopsy suggesting that, in these cases, the detection of circulating cells might be related to the mobilisation of cells linked to the tumor sampling. The presence of circulating tumor cells was not correlated with the size of the primary tumors nor with the presence of metastasis. Ewing cells infiltrating BM were detected in 13/41 patients. The presence of RT-PCR positive BM was most frequently observed in patients with metastasis (8/17) as compared with pts with localized disease (5/24) defined by cytologically negative BM, normal pulmonary CT and bone scan. Nevertheless, half of the patients (9/17) having lung or bone metastasis did not had RT-PCR positive BM. Follow-up of the 5 patients with RT-PCR positive BM and otherwise localized Ewing's tumour should enable to precise the prognostic significance of this marker. During the course of therapy, tumor cells could still be evidenced in the bone marrow after initial chemotherapy in two patients ; both demonstrated rapid clinical progression, suggesting that successive analysis of BM by RT- PCR might be usefull for the monitoring of response to treatment.

O-125

DETECTION OF EWING'S TUMOR CELLS IN BONE MARROW AND PERIPHERAL BLOOD SAMPLES BY MEANS OF RT-PCR. A PRELIMINARY REPORT OF THE EICESS 92 MOLECULAR GENETIC COMMITTEE.

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Long-term disease free survival in Ewing's tumor patients (ET) with localized disease has greatly improved with intensive chemotherapy. However, a subset of patients may relapse due to minimal metastatic dissemination at diagnosis not detectable by conventional staging methods. RT-PCR based detection of Ewing's tumor specific EWS rearrangements was performed with bone marrow and peripheral blood samples to identify minimal metastatic and residual disease.

Patients and Methods: In a European multicenter study (EICESS), bone marrow and peripheral blood samples from *61* ET patients were analysed by RT-PCR to identify chimeric fusion transcripts caused by t(11;22) or t(21;22) translocations. *29* ET pts had localized disease, *20* pts primary metastasis (13 pts. multifocal bone disease, 1 pts. lymphnode, 6 pts isolated lung metastasis); *12* pts were presented at relapse (2 local recurrance, 10 multifocal bone disease).

Results: In 24 % (7/29) of the pts with localized disease translocation specific transcripts were detected in bone marrow samples by RT-PCR, whereas all blood samples were negative. After a median follow-up of 15 month in 1 of these patients a sytemic relapse occurred. While RT-PCR revealed chimeric transcripts in all 23 patients with multifocal bone disease (13 pts at diagnosis, 10 pts at relapse), in 1 pt with lymphnode metastases and 2 pts with local recurrance, light-microscopic examination of bone marrow identified tumor cells in 12 out of these 26 pts (46%). 11 pts (42%) showed PCR positivity in peripheral blood samples. In contrast, only 3/6 pts with isolated lung metastases showed PCR signals in the bone marrow.

Conclusion: These results suggest that bone marrow samples provide the more feasible clinical targets than peripheral blood samples for detecting minimal metastatic or residual disease by means of RT-PCR. Clinically localized diseases can be accompanied by occult bone marrow infiltration, which may be of predictive value for clinical outcome. However, this will require prospective validation with a larger number of patients and a longer follow-up period.

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O-126

FREQUENT LOSS OR REDUCTION OF THE DCC GENE EXPRESSION IN HUMAN OSTEOSARCOMA

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Recently, a putative tumor suppressor gene termed deleted in colon carcinoma gene (DCC gene) has been identified. It is known to code for a neural cell adhesion molecule and maps to the chromosomal region 18q21.3. Since allelotype analyses in human osteosarcomas revealed a frequent allelic loss on the long arm of chromosome 18, the localization of the DCC gene suggests its involvement in the pathogenesis of osteosarcoma.

To test this hypothesis we searched for rearrangements on the DNA level in osteosarcomas and various tumor cell lines using a 1.65kb DCC cDNA probe comprising nucleotides 591 to 2250 of the DCC cDNA sequence. In contrast to several colon carcinoma cell lines, osteosarcomas and osteosarcoma derived cell lines revealed no abnormalities in DNA blots. However, DCC expression studies based on a reverse transcriptase polymerase chain reaction connecting exons 6 and 7 of the DCC cDNA sequence showed a loss of DCC mRNA expression in 14 of 28 osteosarcomas (26 high-grade, 2 low-grade tumors) and in the osteosarcoma cell lines TE 85 and Sa-OS. Moreover, a markedly reduced DCC expression was found in 6 of the 28 tumors and in the U2-OS cell line. In normal human bone tissues DCC transcripts have been consistently detected by an RT-PCR based approach but not by the less sensitive Northern analysis. Studies on the expression of the DCC protein in osteosarcoma are under way to endorse our results.

In conclusion, the DCC gene may be a further candidate in addition to the p53 and retinoblastoma gene, which may contribute to the development or progression of osteosarcoma, perhaps through altered growth controlling cell-cell interactions.

O-127

Prospective study of loss of RB gene Heterozygosity in osteosarcomas of the young: preliminary results of the ongoing study OS 94 of the French Society for Pediatric Oncology.

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In 1994, We presented the results of a retrospective study of RB gene Heterozygosity (RB LOH) in High-Grade Osteosarcomas of the young. This abnormality appeared to be strongly linked to a bad prognosis in these patients. (SIOP San Francisco 1994, published in JCO 1996). In this first study, the DNA was extracted from paraffin-embedded sections of tumoral tissue from the initial biopsy. We then tried to search for the reproducibility of these results in a prospective study, SFOP OS 94 activated in June 1994. From June 1994 until December 1996, 56 patients entered the OS 94 Study. until December 96, we could obtain deep-frozen biopsy material from 17 patients. RB gene is studied by PCR of a polymorphism located in intron 20. LOH is visualized on acylamide gel after silver staining.
Although these are preliminary results, they are similar to those of the previous study: **4 patients have no RB LOH:** 3 of 4 are Good responders and doing well, 1 of 4 is not yet evaluable. All 4 patients are presently doing well. **2 have a lost of the entire locus**: these two patients are in progressive disease or already dead. **11 have RB LOH** : 7 of 11 are bad responders (4 of 7 had metastasis during the first year from diagnosis, and 3 are presently in first complete remission) 1 is not yet evaluable, and 3 are good responders and presently doing well . The median follow-up is very short (6 months), but the early results are comparable to our first small series. This study is in progress and we are simultaneously looking for MDR protein expression.

O-128

STATUS OF THE TUMOR SUPPRESSOR GENE *CDKN2* (*MTS1/INK4A*) AND FUNCTIONALLY RELATED GENES IN THE EWING FAMILY OF TUMORS

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Orderly progression through the cell cycle is regulated by multi subunit protein complexes containing cyclins and cyclin dependent kinases. The tumor suppressor *p16* is an inhibitor of cyclin dependent kinase 4 (*CDK4*), which is associated with cyclin D1 (*CycD1*), and negatively regulates G1-S transition by prohibiting the phosphorylation of the retinoblastoma gene product. The *p16* gene, *CDKN2* (*MTS1/INK4A*), is inactivated by mutation and/or deletion in many human malignancies. Alternatively, amplification and overexpression of *CDK4* and *CycD1*, has been observed in brain tumors and sarcomas. Among neural crest derived tumors, *CDKN2* is altered with high frequency in melanomas but rarely in neuroblastomas. We investigated the status of *CDKN2*, *CDK4*, and *CycD1* genes in the Ewing family of tumors (ET). Fourtyfive primary ET samples and cell lines from 21 patients were studied for the presence of *CDKN2* alterations. A hemizygous nonsense mutation was found in only one case. Among 18 primary tumors analysed on Southern blots, homozygous *CDKN2* deletions were detected in 3 cases. In contrast, 50% of all cell lines lacked *p16* expression due to gene loss. The closely *CDKN2* related *p15* gene was codeleted in all but two cases. *P15* loss without *CDKN2* deletion was never observed. There were no discrepancies in the *CDKN2* status between the primary tumors and the corresponding cell lines and between cell lines established from consecutive tumor samples. Neither amplification nor overexpression of *CDK4* and *CycD1* genes were observed. Our results indicate that *CDKN2* alterations play a role in a minor percentage of ET patients and are involved in selective in-vitro growth or survival properties of the tumor cells.

O-129

CLINICAL RELEVANCE OF p53 GENE MUTATIONS IN HIGHLY MALIGNANT OSTEOSARCOMAS

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Mutations in the tumor suppressor gene p53 most probably play an important role in the oncogenesis of a wide range of malignant tumors. Recent studies have shown that the mutated p53 protein also exerts an influence on anticancer treatment response (Dittmer et al., 1993) by activating multidrug resistance gene-1(MDR-1). Among all other pediatric tumors, the osteosarcomas are those which demonstrate best that an aggressive chemotherapy leads to a better prognosis of the tumor. For our investigations, we therefore defined the number and type of p53 mutations in conventionally intramedullary osteosarcomas. Additionally, it was of interest to find out whether there is a correlation between morphologic response to chemotherapy and alterations in p53 gene.
Fresh material as well as formalin-fixed and paraffin-embedded material of 43 highly malignant osteosarcomas of 40 patients was used, with material of 11 benign osteogenic tumors serving as control.
Screening to detect possible gene alterations was performed by PCR / SSCP analysis and direct sequencing. Altogether, 7 p53 mutations were found in the tumor tissue of 6 patients, constituting 16.3% of the patients examined. 5 of the mutations were missense mutations. A splice mutation lacking 11 base pairs in the p53 gene could be proved in the first biopsy and a lymph node metastasis in one patient. These preliminary data show that p53 mutations could be found predominantly in tumors, which histologically showed no or only poor response in the resection specimens.

O-130

DYNAMIC MAGNETIC RESONANCE IMAGING FOR PREDICTION OF TUMOUR RESPONSE DURING PRE-OPERATIVE CHEMOTHERAPY IN OSTEOSARCOMAS.

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In order to quantify the efficiency of the preoperative chemotherapy (PCT) in osteosarcomas (OSA) clinical aspects, laboratory data and conventional X-rays can only be used with limited value. Parametric bone scintigraphy turned out to be a highly sensitive method but is sophisticated and time consuming and therefore not commonly accepted. We demonstrate our experience with magnetic resonance imaging (MRI) using a paramagnetic contrast agent Gadolinium (Gd)-DTPA) in a dynamic manner for monitoring the local efficiency of PCT in osteosarcoma (n=85) since 1988.
45 patients could be followed up three times preoperatively. After detecting a representative central slice out of all T1 weighted scans through the tumour, Gd-DTPA was applied in a dosage of 0.2 ml/kgbw as a bolus intravenously within 5-8 seconds and a repetitive scanning of this slice was performed in spin-echo-mode with TE=15 and TR=200 msec offering an image every 45 seconds. An "irregular region of interest" (ROI) was used for quantification. A phantom was introduced as a standard. The time intensity diagram reflects the significant increase and high plateau phase indicating a hyperperfusion of a malignant tumour. Due to successful PCT perfusion decreases and the signal intensity declines correspondingly, indicating a responder. In optimal situations the dynamic MRI was performed prior to biopsy, after 6 weeks and preoperatively. Considering that the technical and biological margin of error of dynamic measurements is +/- 10%, we defined a decrease of Gd-DTPA perfusion of more than 20% in after 6 weeks and 50% at the end of PCT as an indicator of a responder. In doing so and comparing all resected specimens concerning their histological mapping of remaining viable tumour areas to our dynamic results, the diagnostic accuracy of presurgical prediction of tumour response was $\geq$.80 - $\leq$.90 ($p < .001$).

O-131

MONITORING NEOADJUVANT CHEMOTHERAPY IN PATIENTS WITH HIGH-GRADE BONE SARCOMA USING PERFUSION-SENSITIVE IMAGING TECHNIQUES

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Monitoring preoperative chemotherapy in patients with high-grade bone sarcoma has critical consequences for strategy and timing of surgery and radiation therapy. Particularly early identification of poor respondents is clinically relevant. Evaluating the most cost-effective, noninvasive diagnostic measures aids in the design of prospective trials in the treatment of those patients. Conventional radiography, color Doppler ultrasonography (CDUS), three-phase bone scintigraphy and dynamic contrast-enhanced MRI were applied to monitor chemotherapy in 40 patients with osteogenic or Ewing's sarcoma. Imaging data were compared with the postsurgical histologic response. Plain radiographs and bone scintigraphy were unreliable in predicting response to chemotherapy. Tumor (neo)vascularization and perfusion appeared to be principal targets in monitoring chemotherapy. Persistent intratumoral flow with decreased resistive index, measured with CDUS, indicated poor histologic response after two cycles of chemotherapy in bone sarcomas with an associated soft-tissue mass ($p = .03$). Fast dynamic contrast-enhanced MRI was superior in monitoring efficacy of therapy in (small) intraosseous tumours and preoperative local staging. A short-time interval of < 6 sec between arterial and tumoural enhancement strongly suggests residual viable tumour. Even small clusters of remnant viable tumour could be identified after therapy at well-known sanctuary sites. We therefore conclude that the combination of CDUS and MRI is superior for monitoring efficacy of therapy in patients with bone sarcoma.

O-132

^{31}P MRS OF HUMAN MUSCULOSKELETAL TUMORS IN RESPONSE TO CHEMOTHERAPY

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Objective: to evaluate the potential of ^{31}P magnetic resonance spectroscopy (MRS) for an assessment of the response to pre-operative chemotherapy in patients with malignant bone and soft tissue tumors. Three types of protocols were compared, utilizing (i) only pre-therapeutic metabolite ratios, (ii) changes in spectroscopic parameters after the first cycle of chemotherapy, and (iii) long-term changes during therapy. — **Methods:** 29 patients were examined at 1.5 T by means of ^{31}P MRS and surface coils (FROGS, CSI). Tumor changes during chemotherapy were monitored in 22 cases. Spectroscopic parameters were compared to the percentage of tumor necrosis determined from pathologic evaluation after surgery (10 responders, 19 non-responders). — **Results:** Tumor spectra typically showed elevated signals of low energy phosphates (LEPs) and a decrease in high energy phosphates (HEPs). PCr/α-ATP was significantly reduced in pre-therapeutic spectra of non-responders (1.15 ± 0.56 vs. 1.80 ± 0.51, $p < 0.03$), indicative of poor oxygenation. Responders normally showed a marked increase of LEPs after the first cycle due to therapy-induced cell-killing, followed by a regression of LEPs/HEPs. This ratio progressively increased in non-responders. Significant differences were obtained comparing changes in LEPs/HEPs during therapy between responders and non-responders: short-term changes, 5.9 ± 6.1 %/d vs. 0.7 ± 3.2 %/d, $p < 0.05$; long-term changes, -0.77 ± 0.30 vs. 1.1 ± 1.0 %/d, $p < 0.002$. — **Conclusion:** ^{31}P MRS is a promising tool for monitoring tumor treatment outcome.

O-133

POSITRON EMISSION TOMOGRAPHY [PET] SCANNING WITH ^{18}FLUORODEOXYGLUCOSE [FDG], CORROLATES WITH HISTIOLOGICAL RESPONSE IN CHILDREN WITH OSTEOSARCOMA [OS] AND EWINGS SARCOMA .[ES]

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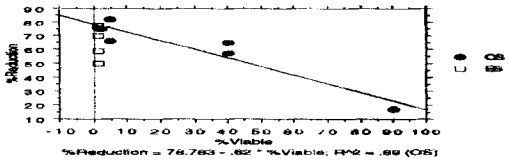
Current imaging modalities are inadequate for measurement of tumor viability post-chemotherapy in children with OS or ES. Glucose metabolic rates as determined by FDG PET has the potential to distinguish viable from non-viable tumors. Therefore, we undertook a prospective sequential study of FDG PET imaging in children with OS [n=7] and ES [n=5]. FDG, 0.14mCi/Kg [10mCi max] was injected after 4 hours fast, and whole body PET images were obtained. FDG uptake was obtained by placing identical regions of interest over the tumor and contralateral normal side, and a tumor/normal ratio was derived. Pre- and post-treatment FDG ratios were compared and percent reduction determined. Maximum percent reduction in FDG was compared with extent of viable tumor by histopathology of the resected tumor (see Figure).



In the 7 OS patients who underwent resection, the % reduction in FDG uptake [Range 17-82%] correlated inversely with % viable tumor [<1-90%], r=0.89, p=<0.01. All ES patients had less than 1% viable tumor at resection and thus no correlation could be established with % reduction in FDG. These preliminary observations suggest that sequential PET FDG may represent an important clinical tool in the evaluation of children OS and merits further study in children with ES.

O-134

DEFECTIVE EXPRESSION OF THE GM-CSF/IL3/IL5 RECEPTOR COMMON β CHAIN IN AML

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Pulmonary alveolar proteinosis is a cause of fatal respiratory failure with heterogenous etiologies which may be genetic or aquired. Some cases of PAP have been reported to be associated with hematologic malignancies such as AML. In mice, we have generated the PAP phenotype by targeted deletion of the gene for the GM-CSF/IL3/IL5 receptors common β chain (βc). In βc −/− animals steady state hematopoiesis was normal except for lower eosinophils. In addition, we observed a slower hematopoietic recovery following transplantation of βc mutant bone marrow into wild type recipients. The fact that βc is almost exclusively expressed in myeloid cells provides evidence for a causal relationship between the lung disease and the hematopoietic system. We have identified a mutation and expression defect of βc in congenital PAP in humans. Here, we describe an expression defect of βc in two pediatric patients with congenital AML and PAP-symptoms. The patient`s blasts failed to express normal levels of βc as shown by flow cytometry. Strikingly reduced or absent function of βc was demonstrated in clonogenic progenitor assays with absent CFU growth following GM-CSF or IL3 stimulation. The response to growth factors acting via receptors distinct from βc (IL6/SCF or G-CSF) was normal. Following anti-leukemic treatment the pulmonary symptoms resolved and βc expression was normal. Our findings provide evidence that a defect in the expression of βc AML blasts can be associated with pumonary insufficiency in patients with AML.

O-135

EXPRESSION OF THE EVI-1 GENE IN MYELOID CLONOGENIC CELLS FROM JUVENILE CHRONIC MYELOGENOUS LEUKEMIA (JCML)

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The ecotropic virus integration site-1 gene (EVI-1) encodes a zinc-finger DNA binding protein whose aberrant expression has been associated with impaired granulocytic differentiation and abnormal response of erythroid precursors to erythropoietin. Expression of EVI-1 occurred in patients with myelodysplastic syndromes (MDS) and in acute leukemia with and without 3q26 translocations. More recently constitutive EVI-1 gene transcription has been found in peripheral blood cells of juvenile chronic myelogenous leukemia (JCML) patients. In order to investigate which cell types are responsible for the EVI-1 aberrant expression in JCML leukemic cells, we analysed its level of transcription in JCML myeloid clonogenic cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of single CFU-GM colonies obtained in vitro in the absence of added growth factors from peripheral blood mononuclear cells of five JCML patients, detected EVI-1 expression only in a minority of the tested colonies (3/22; 13.6%). On the contrary most of the CFU-GM colonies obtained from highly purified (>95%) CD34-positive cells, were positive for EVI-1 transcript, suggesting that its expression is restricted to more immature progenitor cells. Similarly, the leukemic cells occurring during blast crisis of one JCML patient displaying the typical features of a stem cell leukemia (CD34+, CD19-, CD2-, myeloperoxidase-), expressed EVI-1 mRNA. When normal human bone marrow or cord blood were assessed for EVI-1 gene expression, only few CFU-GM colonies were found to be positive. Our findings suggest that inappropriate expression of transcription factors (such as EVI-1 or GATA-1) linked to early stages of differentiation, may represent the neoplastic nature of JCML CD34+ clonal progenitor cells.

O-136

STRUCTURAL AND NUMERICAL ABERRATIONS OF CHROMOSOME 7 IN CHILDREN WITH ALL, AML, AND MDS*

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Chromosomal abnormalities involving chromosome 7 are frequent in all types of hematological malignancies and the loss of the entire chromosome (-7) or parts of the long arm (7q-) are known to be an indicator of poor prognosis in AML and MDS. Analyzing bone marrow and blood samples of children with ALL, AML, and MDS a chromosomal abnormality involving chromosome 7 was detected in 139 patients: 70 with ALL, 47 with AML, and 22 with MDS. Comparing the breakpoints of 7q in these 3 groups, we found that in ALL the breaks occur mainly in the proximal part of 7q (bands q10-q11:19/31pts =61%), whereas in AML and MDS a break in these bands could be detected in only 5/16 (31 %) and 1/4 (25%), respectively. In the latter groups, however, breaks in the middle or distal parts of the long arm predominated: q22-q36:11/16 and 3/4 (69 and 75%). These regions were less frequently involved in ALL:12/31 (39%). Several types of chromosomal abnormalities could be detected in the 3 diagnostic groups: In MDS-patients a monosomy of chromosome 7 (-7) predominated (18/22 =82%) and only a few other aberrations appeared, which all led to a loss of a distal part of the long arm (7q-). More than half of the children with AML showed a loss of chromosomal material caused by -7 (14/47 =30%), del(7q) (7/47 =15%), an unbalanced translocation (3/47 =6%) or a ring chromosome (1/47 =2%). In ALL-patients, however, gain of chromosomal material due to isochromosomes (i(7q): 39%) or duplications (5.6%) was most frequent, whereas a complete loss of the whole chromosome 7 or a part of the long arm is less frequent in this group. It was detected in only about ¼ of the children. Cytogenetic analyses were completed by FISH. It will be shown that this technique is needed using centromeric probes for the screening of MDS and specific YACs for the breakpoint analysis of the long arm.

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O-137

OVEREXPRESSION OF SHC PROTEINS: ANOTHER POTENTIAL MECHANISM RESPONSIBLE FOR GM-CSF HYPERSENSITIVITY IN JUVENILE CHRONIC MYELOGENOUS LEUKEMIA (JCML)?

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The exquisite hypersensitivity to GM-CSF of the JCML bone marrow (BM) or peripheral blood (PB) progenitors is one of the consistent abnormality demonstrated by in vitro studies in JCML patients. When highly purified preparations of PB and BM CD34-positive cells from a JCML patient are cultured in semisolid media, a dramatic difference in the dose-response curve for CFU-GM with increasing concentrations of GM-CSF is observed when compared to normal BM CFU-GM growth. Although the molecular basis of GM-CSF hypersensitivity are still unknown, several evidences indicate that the GM-CSF signal transduction pathways might be altered. Consistent with previous findings, mutation in the N-Ras gene have also been found in 4 out of 11 patients with JCML. Nucleotide sequence analysis showed single nucleotide substitutions involving codons 12 (GGT→TGT) and 13 (GGT→GAT) in exon 1 and codons 61 in exon 2 (CAA→CTA). The Ras point mutations may lead to elevated levels of Ras-GTP, resulting in deregulation of GM-CSF signal transduction pathway. Among the different components of Ras signalling pathway, Shc proteins have been shown to play an important role in the proliferative response to GM-CSF, being phosphorylated and complexed with the β-submit of the GM-CSF receptor upon GM-CSF stimulation. In order to investigate the potential role of the Shc proteins in a GM-CSF human factor-dependent cell line, Shc cDNA was introduced into GFD8 cells by retroviral mediated gene transfer and clones overexpressing Shc proteins were selected. The growth response of SHC and SN GF-D8 clones to GM-CSF was analysed by the soft agar clonogenic assay. The clones which displayed the maximum levels of Shc proteins displayed GM-CSF dose response curves comparable to the ones commonly observed in JCML patients. Based on these findings, data are in progress to evaluate the potential role of Shc proteins (overexpression or constitutive phosphorylation) in the selective GM-CSF hypersensitivity displayed by JCML BM/PB progenitor cells.

O-138

CLINICAL SIGNIFICANCE OF DETECTION OF *AML1/ETO* FUSION mRNA IN PATIENTS WITH t(8;21) ACUTE MYELOID LEUKEMIA

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The chromosomal translocation t(8;21)(q22;q22) in acute myeloid leukemia (AML) can be detected by a reverse transcription-polymerase chain reaction (RT-PCR) for the chimeric *AML1/ETO* transcript. The clinical significance of minimal residual disease (MRD) is still a controversial issue. To assess it, we used RT-PCR to analyze peripheral blood (PB) and bone marrow (BM) samples from eleven patients who reached a complete hematological remission after chemotherapy and bone marrow transplantation (BMT). In BMT group, four patients were treated with allogeneic BMT including one second BMT, and two were autologous BMT. Other six patients were treated with chemotherapy currently used in our institute. PB and/or BM samples were taken at the time of diagnosis, and during and after the therapy. Total RNA was prepared from one to 10×10^{6} cells with the acid guanidinium thiocyanate-phenol-chloroform method. The first-strand cDNA was synthesized from one microgram RNA by reverse-transcriptase, and 1st step PCR was performed using one-eighth of the cDNA , and using the one-tenth of 1st step PCR product nested PCR was performed. One half of each 1st step PCR and nested PCR products was run on agarose gel and detected with ethidium bromide. The detection limit of the first step PCR was 1 in 10^{3} RNA of cells, while nested PCR was 1 in 10^{5}. In the BMT group, the MRD became negative rapidly in four cases and they have not relapsed for 29, 45, 49, and 52 months. But the other two cases, MRD remained positive after BMT, and they relapsed soon. In the chemotherapy group, in four patients MRD was disappeared during chemotherapy or for one year after therapy, and they have sustained clinical remission from two to four years. But two patients, MRD was detectable for long term. One patient is in CR for 55 months, but the other was relapsed during the chemotherapy. Contrary to previous studies on MRD associated with t(8;21)AML indicated that the *AML1/ETO* fusion transcript was positive in most patients even in long-term remission, our results indicated a better correlation between the results of RT-PCR assay for *AML1/ETO* expression and prognosis of patients. This study suggests that if its sensitivity is appropriate, the detection of MRD is a useful tool for monitoring MRD and selecting treatment in t(8;21)AML

O-139

EXPRESSION OF THE EVI-1 GENE IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) IN CHILDHOOD

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The EVI-1 gene was identified as the gene associated with a common site of retroviral integration in murine myeloid leukemias. It encodes a DNA binding zinc finger protein of 145 kDa which is believed to block granulocytic differentiation in response to G-CSF while it does not influence the monocytic proliferation and differentiation. In addition, the expression of the EVI-1 gene impairs the normal response of erythroid precursors to erythropoietin. For this profile we investigated the expression of EVI-1 in leukemic cells of 15 children with CMML. We examined mononuclear cells of peripheral blood (n=8) and bone marrow (n=9) by RT-PCR. As controls peripheral blood (n=10) and bone marrow cells (n=6) from healthy donors were studied. cDNA (up to 100 ng) was subjected to PCR with 35 cycles. PCR-products were Southern blotted. The expression of the EVI-1 gene was evaluated relatively to ß-actin. In contrast to other published reports we detected a constituous level of EVI-1 transcription in mononuclear cells of healthy donors with similar levels of expression in peripheral blood and bone marrow cells. In comparison to controls higher levels of EVI-1 expression were seen in bone marrow cells of 4 (44%) and peripheral blood cells of 4 (50%) CMML-patients. In two cases peripheral blood and bone marrow cells of the same patient were examined. In one case high EVI-1 levels were found in both specimens, in the other case increased expression was only seen in peripheral blood. Further investigations need to determine which cell types are responsible for the increased expression of EVI-1 in leukemic cells of some children with CMML.

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O-140

DOWN´S SYNDROME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: CLINICAL CHARACTERISTICS AND TREATMENT OUTCOME IN FOUR CONSECUTIVE BFM TRIALS

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Of 4110 children with acute lymphoblastic leukemia (ALL), treated during four consecutive trials (March 1981-December 1994) of the Berlin-Frankfurt-Münster group (BFM), 61 (1.5%) had Down´s syndrome (DS). Sex ratio, white blood count and leukemic cell burden were comparable to ALL children without Down´s syndrome (NDS). Patients with DS tended to be older ($p<0.05$), lacked CNS disease (0% vs 2.5%) and were significant less likely to present with an anterior mediastinal mass (1.6% vs 8.7%, $p<0.05$). In DS patients, comparison of major immunophenotypes revealed a predominance of c-ALL (80% vs 65.2%, $p<0.05$) and absence of T-ALL (0% vs 13.4%, $p<0.001$). The frequency of hyperdiploidy (DNA-index ≥ 1.16) was significantly lower in the DS cohort than that in the general ALL population (6.7% vs 25.8%, $p<0.02$). Initial treatment response to prednisone was better in the DS group (prednisone good response in 98.1% vs 90.7%), but did not reach statistical significance ($p=0.057$). Despite predominance of favorable prognostic features, the DS group fared worse as compared to the NDS controls (pEFS 0.58 vs 0.70, $p=0.14$). Bone marrow relapse was the leading cause of death in DS patients (70% of all events). Serious treatment toxicity, especially due to Methotrexate, and infectious complications occured more frequent among children with DS and caused death in 4 patients (6.6%). However, with a median observation time of 5.1 years (range 1-14 yrs), the pEFS for DS children who received treatment with no major modifications (57%) was similar to the NDS controls (pEFS 0.65 vs 0.70, $p=0.66$). Both, suboptimal treatment and infectious problems contributed to the inferior survival. These findings strongly suggest that ALL patients with Down´s syndrome should receive intensive protocol treatment without major modifications, but require more effective management of toxicity.

O-141

Results of the DCLSG[1])-study ALL 7: the Dutch experience with BFM-oriented treatment in children with ALL (1988-'91).

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In the DCLSG study ALL 7 chemotherapy and treatment stratification into 3 risk groups, based on tumor mass and early response to treatment (standard risk (SRG), risk (RG), experimental (EG)) were identical to ALL-BFM 86 (Reiter et al. Blood 1994;84:9122-33), but cranial irradiation was restricted to pats. with initial CNS-involvement (± 2%). As in ALL-BFM-86 a randomized study on late intensification (Prot.S) was performed in RG-pats. and during the study early reinduction treatment (Prot.II) was introduced for SRG-pats. Treatment duration (all pats.): 18 months. Accrual period: 7/'88 - 10/'91.
Results: 218 pats. were entered: 74 SRG-, 127 RG- and 17 EG-pats.
Overall CR rate: 97%. EFS (at 5 years): for all pats. 65% (SD±3), for SRG pats. with and without Prot.II, 73% (SD±8) and 57% (SD±7) resp, and for RG- and EG pats. 66% (SD±4) and 63% (SD±12) resp. Sixty three (29%) pats. relapsed: 24 (11%) during and 39 (18%) after therapy. The number of BM, CNS, combined BM+CNS and other extramedullary relapses was 45 (SRG 17, RG 27, EG 1), 12 (SRG 5, RG 7, EG 0), 3 (SRG 1, RG 2, EG 0) and 3 (SRG 2, RG 1, EG 0) resp. In SRG pats. the CNS-relapses occurred only in pats. without early reinduction treatment with Prot.II. Six children died in CCR, 2 suffered from a second malignancy. No difference in EFS was observed in RG pats. treated with or without Prot.S (study limited to 51 pats.).
Conclusions The results of DCLSG ALL7 confirm those of ALL-BFM 86:
1. early reinduction treatment (Prot.II) is essential for SRG-pats.
2. late intensification (Prot.S) does not improve the treatment results in RG-pats.
Considering the late effects of cranial irradiation it is our judgement that children with ALL without initial CNS involvement can be treated with BFM-oriented treatment without radiotherapy.

O-142

THE CLINICAL SIGNIFICANCE OF BCR-ABL IN RELAPSED CHILDHOOD ALL A MATCHED PAIR ANALYSIS

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Background. The Philadelphia chromosome (Ph^1) or its molecular equivalent the expression of BCR - ABL - mRNA is one of the most striking and well-characterized cytogenetic abnormalities in leukaemia. But it still remains unclear whether the Ph^1 is an independent risk factor for outcome of leukemia or not. It has been shown that relapse Ph^1 positive acute lymphoblastic leukemia (ALL) is associated with a higher rate of drug - resistant leukemia, shorter duration of remission, higher white blood cell count, higher blast cell count, and higher age.
Methods. To this end a matched pair analysis was performed within a very homogeneous group of patients. That is children presenting with a first relapse of ALL treated according to ALL-REZ BFM protocols. Alltogether 307 patients were eligible for this analysis, 30 positive and 277 negative, respectively. Positive patients were matched exactly for time point of relapse (on or off therapy), location, immunophenotype, and matched as close as possible for duration of first remission, peripheral blast cell count, white blood cell count, and date of therapy onset.
Results. Univariate survival analysis resulted in a probability of event-free survival at 5 years of 0.451 for negative and 0.084 for positive patients, respectively ($p = 0.0009$). Multivariate analysis using the Cox model revealed risk ratios of 4.462 for relapse on therapy, 3.344 for Ph^1 and/or expression of BCR - ABL - mRNA, 1.584 for high peripheral blast cell count, and 0.275 for bone marrow transplantation.
Conclusions. Thus it could be shown that the Ph^1 is indeed an independent risk factor in childhood relapsed ALL. There are striking similarities between positive patients at relapse and at initial diagnosis as well as adult patients. Therefore it is highly suggestive that the Ph^1 is also an independent risk factor under all these circumstances.

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O-143

Ph[1] CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: IDENTIFICATION OF SUBSETS AT DIFFERENT PROGNOSIS

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9;22 translocation (Ph[1]) is considered one of the worst prognostic factors for childhood acute lymphoblastic leukemia (ALL). Recently a few cases with unexpectedly favorable treatment response have been described. In order to identify possible subsets of Ph[1] childhood ALL we retrospectively evaluated the AIEOP data-base of the years 1988-94. Presenting features and treatment response were evaluated.

Twenty-six pts (19 males) were identified; the median age was 7 yrs (range 0-15 yrs); median WBC count was 65.000/mm^3 (range 3.800-572.000); immunophenotype was common (n=14), pre-B (n=5), pre-pre-B (n=1), AHL (n=6). All pts were enrolled in current high risk protocol (8803 n=8, 9103 n=15) except for 3 enrolled in the intermediate risk protocol (8702, 8802, 9102). 15 of 24 evaluable pts were prednisone good responders (PGR) (62%).

Of the 26 pts 10 (38%) had died (8 with active disease), 16 (62%) were alive: 12 (46%) in CCR (7 after BMT) at a median time of 32 months (range 10-82 mos), and 4 with disease. Among the 15 PGR pts 4 of the 9 treated with chemotherapy (CT) only, and 5 of the 6 who had also BMT, were in first remission; of the 9 non-PGR pts 7 were resistant to induction therapy, one relapsed and one is in remission after BMT. Of the 11 pts presenting with WBC <50.000/mm^3, 8 were 1 to 9 yrs old and all these 8 were PGR: 7 of these 8 remain in first remission after CT only (at 10+,13+,57+ and 82+ mos, with one relapse at +11) or CT+BMT (at 23+, 32+, 35+ mos).

In this restrospective study, age, WBC, immunophenotype were not associated with the outcome. Yet, we identified two subsets of Ph[1] ALL with different prognosis: non-PGR pts remain at extremely high risk for treatment failure, particularly for induction failure; on the contrary the association of PGR, age 1-9 yrs, and WBC <50.000/mm^3 identifies pts at remarkably lower risk of relapse after BMT or intensive CT.

O-144

TOXICITY, SUPPORTIVE CARE, AND COSTS OF TWO CHEMOTHERAPY PROTOCOLS FOR TREATMENT OF CHILDHOOD ALL IN RUSSIA: BFM90 AND MB91

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Since 1991, a single-center study with two chemotherapy protocols BFM90 and MB91 was performed in Moscow. MB91 consisted of a four-agent induction therapy including dexamethasone, consolidation therapy including weekly L-asparaginase, prolonged intrathecal triple chemotherapy, and conventional maintenance therapy with reinduction pulses. As previously reported, event-free survival rates at three years were comparable for both protocols.

Aim of this study was to compare toxicity, need of supportive care and expense of both regimens (BFM 25 pts; MB 32 pts). During induction and consolidation phase, toxicity was regularly assessed by measurements of blood counts, bloodchemistry, and clinical parameters. Cytostatic and supportive therapy has been documented for each patient. Hepatotoxicity (liver enzymes), nephrotoxicity (creatinine), duration of neutropenia, and thrombocyte substitutions were similar in both protocols. The need of erythrocyte transfusion was different in BFM compared with MB (1000 vs 505 ml/m², p<.01), as well as IV antibiotic therapy (22 vs 9 days, p<.01), treatment delays (39 vs 21 days, p<.001), and duration of in-patient treatment (47 vs 18 days, p<.001). Side effects of the MB protocol mainly occurred during induction therapy. Total costs of treatment including supportive care were 1.7-fold higher in BFM than in MB, whereas costs of cytostatic drugs were comparable in both groups.

Conclusion: In Russia, both protocols were feasible. Tolerance to treatment was better in MB91 compared to BFM90. However, intensity of induction therapy was similar in both protocols, possibly due to the use of dexamethasone in MB. With respect to costs and side effects, the MB91 protocol appears to be an alternative to established protocols in countries with limited financial and clinical resources.

O-145

THE ROLE OF CRANIAL IRRADIATION (CRT) FOR CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) WITH HIGH LEUKOCYTE COUNT AND PREDNISONE GOOD RESPONSE (PGR).

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BFM ALL-90 and AIEOP ALL-91 studies share the same treatment backbone and have a superimposable 30 months overall EFS. Both groups, BFM and AIEOP, have recently described PGR (<1000 blasts after 7-day steroid therapy) as a powerful prognostic factor in childhood T-ALL. To further refine treatment strategy, in this intergroup study we investigated the role of CRT in PGR T-ALL pts according to leukocyte count and BFM risk factor (RF).

138 pts (106 males, median age 7.5 years) with T-ALL, PGR, RF ≥0.8 <1.7 and no CNS leukemia, diagnosed between 1990 and 1994, were enrolled in the BFM ALL-90 (n=81) or in the AIEOP ALL-91 (n=57) studies, respectively. All pts received the "BFM backbone": 8-drugs induction, 5 gr/m^2 methotrexate consolidation, reinduction and maintenance. CNS directed therapy consisted of CRT and IT-MTX (11 doses) in the BFM study and of extended triple intrathecal (TIT, 17 doses) in AIEOP study. Pts were analyzed according to leukocyte count (cut-off: 100.000/mm^3). EFS (SE) of the two groups was compared using the log-rank test.

For pts treated with CRT (BFM group), 3-yrs EFS was 86.3% (8.1) in 64 pts with leukocyte count <100.000/mm^3 vs 76.1% (12.3) in 17 pts with higher leukocyte count (p=0.04). Conversely, for pts treated without CRT (AIEOP group), EFS was 81.9% (6.8) in 45 pts with leukocyte count <100.000/mm^3 vs 16.7% (13.6) in 12 pts with high leukocyte count (p<0.001). Out of these last 12 pts, one died during induction, 4 relapsed in the marrow and 4 in the CNS during treatment.

T-ALL PGR pts may achieve very good treatment results, exceeding 75% EFS, following intensive BFM based chemotherapy. These data, although based on limited pts number, suggest that in these pts CRT may be safely replaced by extended TIT only if initial leukocyte count is <100.000/mm^3. These data describe subsets of T-ALL pts at different prognosis and further support the hypothesis of an intriguing, significant contribution of CRT to better disease control.

O-146

FUNCTIONAL OUTCOME FOLLOWING LIMB SALVAGE SURGERY ABOUT THE KNEE

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We reviewed the functional outcome of limb salvage surgery about the knee following resection for bone sarcoma in the pediatric population. All patients (pts) were reconstructed utilizing a custom endoprosthesis. All procedures were performed at a single institution and by the same surgical team. 29 limb salvage surgeries about the knee were performed between 1981-1995. There were 14 males and 15 females, median age 14 yrs (range 7-20 yrs). Diagnosis included osteosarcomas (n=26), Ewings sarcoma (n=2), and malignant fibrous histiocytoma (n=1). 23 pts presented with stage IIB lesions, 6 with stage III. Primary sites were distal femur (n=23), proximal tibia (n=6). 3 pts received a Lewis expandable prosthesis, 26 a Lacey custom rotating hinge total knee prosthesis. All pts were evaluated for functional results and radiographically using the I.S.O.L.S. scoring system. Of the original 29 pts, 20 are alive and 15 continue to have a functioning prosthesis. 5 pts had a functioning prosthesis at the time they succumbed to their disease. 10 pts required amputation: 6 for infection, 3 for loosening, and 1 for local recurrence. 3 pts with loosening opted to have their prosthesis replaced. Followup was 30 mos (range 6-84 mos). Functional evaluation scores averaged 66 (range 0-100) for all pts at last exam. For those pts with a functional prosthesis still in place currently or at the time of their death (n=19), the average functional score was 80. Limb salvage surgery for malignant tumors about the knee utilizing a custom endoprosthesis in children and adolescents can yield predictable outcomes with acceptable functional results.

O-147

COMPARISON OF THREE DIFFERENT RECONSTRUCTION PROCEDURES AFTER RESECTION OF MALIGNANT TUMORS CLOSE TO THE KNEE

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Prosthetic arthroplasty, osteoarticular allograft reconstruction and allograft arthrodeses are well established methods for limb salvage surgery in tumors close to the knee. We tried to compare this three methods regarding complications causing revision surgery and functional results. Mean age at primary tumorresection was 16,5 years (range 12 to 33).

47 Patients underwent wide tumor resection and recieved limb salvage of the leg. The proximal tibia was resected 18 times, distal femur was involved 29 times. 19 prosthetic arthroplasties, 15 allograft arthrodeses and 13 osteoarticular allografts were implanted. 89% of the patients received chemotherapy. Complications leading to operative revision were analysed and the functional results were scored using the functional evaluation system of Enneking. The mean follow up was 29 month (range 11 to 70).

All reconstruction procedures show high incidence of complications needing operative revision (50% of prosthetic arthroplasty, 46% of osteoarticular allograft, 42% of allograft arthrodeses). The most serious complication was Infection (18% for prosthetic arthroplasty, 15% for osteoarticular allografts and 16% for allograft arthrodeses). The functional results on average for prosthetik arthroplasty were 84%, for osteochondral allografts 61% and for allograft arthrodeses 66%.

Complication rates and functional evaluation of this three reconstruction procedures show too little differences to prefer one of them. May be longer follow ups will show more significant data.

O-148

Active tibial and femoral titanium growing prostheses in limb sparing salvage for children's bone sarcomas (about ten years experience and 25 patients).

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New techniques in care of children with malignant bone sarcomas have contributed to the increased length of survival. To improve the quality of life becomes now a priority. Growing young children have until recently fared better with an amputation than with limb saving reconstruction because of the unavoidable limb length discrepancy. After Sneath, then Lewis, we tried to use an expandable and adjustable prosthesis. We review our ten years experience with successive models, the last growing without open surgery.

Methods : Prostheses are produced in titanium, chosen for its better mechanical properties, twice as elastic and light as stainless steel. The prosthesis is manufactured following the recommendations of the surgeon for each patient with individualised size. The prosthesis is done with three elements : one special growing part, one epiphyseal part, one tibial or femoral stem. The size of the epiphyseal part is small enough to be inserted from age 5 y and its smooth edges avoid any soft tissue damage. The lengthening of the prosthesis is performed when the discrepancy is ≥ 3 cm. The increase of the prosthesis has no limits, can be > 15 cm, even if the resection was inferior to 15 cm.

Patients : from 1984 to 1995 we used 25 growing prostheses for children aged 4,5 y to 13 y. 3 tibial growing prostheses, 2 superior femoral prostheses, 5 total femur replacements, 15 inferior femur prostheses. The patients had Ewing's sarcoma (6) or osteosarcomas (19).

Results : Five patients died from the illness. 21 had increasing of the prosthesis. The mean lengthening was 6,2 cm (min. 24 mm max. 120). The function was much improved by the lengthening. Only two patients received the definitive prosthesis. Following EMSOS criteria, functional results are rated : excellent or very good (15), fair (6), bad (4). Three patients experimented an infection following the operation to increase the limb, leading to out put the prosthesis to treat infection before revision operation. One was amputated. Two had a new reconstructive surgery.

Conclusion : **The expandable prosthesis has provided an excellent alternative to amputation in young children**. Nevertheless, the infection risk appended to multiple surgical procedures leads us to develop a new generation of growing prostheses which does not need open surgery for lengthening.

O-149

GROWING ENDOPROSTHESIS FOR PRIMARY MALIGNANT BONE TUMOURS

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Twenty years experience of the use of growing endoprostheses for limb salvage in skeletally immature patients is reported. Alternatives for the management of primary malignant bone tumours in this group of patients are amputation, rotationplasty, and allograft or autograft reconstruction. The development of the currently used implant is charted, with commentary on the complications and difficulties which gave rise to the changes made. Over this period 123 expanding endoprostheses have been inserted in 108 patients in this institution, and the results of a series of 54 consecutive distal femoral replacements are reported. The mean functional score was 72% and the local recurrence rate was 11%. The technique was successful in maintaining limb-length equality. We conclude that in specialised centres of orthopaedic oncology, utilising an endoprosthesis with proven reliability of the expansion mechanism, the results of this technique in both the long and short-term, justify its continued use.

Key Words: Expandable Endoprosthesis, Osteosarcoma, Ewing's sarcoma.

O-150

ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANT (BMT-al and ABMT) IN ACUTE LYMPHOBLASTIC LEUKAEMIA. RESULTS IN 128 CHILDREN.

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Indication of BMT-al in ALL in children is restricted to a small proportion (10-12%) of very high-risk patients in first remission (CR1),to patients in 2nd remission (CR2) and those with more advanced disease (AD);the role of ABMT in all these cases has not been well established .The 10 year (1985-1994) experience of a BMT Paediatric Unit is presented. **Patients**.128 patients between 4 months and 16 years old received a BMT, 58 a BMT-al from a HLA-identical sibling and 70 an ABMT.The disease status at transplant was: **a)** in **CR-1**,32 patients:11 had not attained remission after 5 weeks of treatment,7 were phenotype B,2 had t(9;22),2 had t(4;11),2 were mixed lineage AL,2 were infants with WBC>100x10^9/l and 6 had WBC>200x10^9/l and other risk factors. **b)** in **CR-2**, 75 patients:in 82% the first remission was shorter than 36 months.**c)** In **AD**,21 patients:16 in CR3 and 5 in CR4 or partial remission. **Methods**.Pre-BMT cytotoxic treatment consisted on Cyclo/120 mg/kg and TBI, (14 Gy in 7 fractions) with addition of HD Ara-C or VP-16 in most cases.In 87% of ABMT,the marrow was "purged" with MoAb.For GVHD prevention,CSPA was given.**Results:a) CR1**:15 patients received a BMT-al and 17 an ABMT.The EFS,relapse (REL) and transplant related mortality (TRM) were 67%, 20% and 13% respectively for the BMT-al and 52%, 41% and 6% for the ABMT.**b) CR2**.For the 28 patients receiving a BMT-al the SLE,REL and TRM probabilities were 39%, 43% and 18% and for 47 having an ABMT were 43%, 53% and 2% respectively.Results were better if the first remission was longer than 18 months.**c) AD**.EFS,REL and TRM rates were 20%, 46% and 31% for the 15 patients with a BMT-al; only 1 of the 6 receiving an ABMT was alive after 3 years. **Conclusions.**1.BMT-al and probably also ABMT appear as effective treatments for very high-risk ALL children in CR-1. 2.For patients in CR-2 both modalities of BMT showed a similar efficacy in this series; the higher relapse rate in ABMT was compensated with a very low TRM. 3.BMT-al offers a hope of cure for some patients with advanced disease.

O-151

ALLOGENEIC BONE MARROW TRANSPLANTATION FOR CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN THIRD REMISSION: A CONCEIVABLE ALTERNATIVE

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Purpose: After a first bone marrow (BM) relapse of childhood acute lymphoblastic leukemia (ALL); long-term remissions have been reported in 35% of patients with salvage chemotherapy. For 15-25% of children HLA-matched related donors are available, and allogeneic bone marrow transplantation (BMT) can be used for treatment intensification. Aim of this study was to assess the value of BMT performed either in second (CR2) or third complete remission (CR3).

Methods: In the BFM Relapse Study registry, we retrospectively identified 136 patients with BMT in CR2 (strategy A) and another 33 patients who received chemotherapy at first relapse and BMT not before a second bone marrow relapse had occurred (strategy B).

Results: Event-free survival (EFS) rates at 6 yrs after BMT were 0.49 ± 0.05 and 0.48 ± 0.09 for patients with strategy A and strategy B, respectively. In total, 52 of the 169 children (31%) had relapsed, 28 (17%) died of therapy-related complications, 2 (1%) were lost to follow-up, and 87 (51%) are in continuous CR at a median observation time of 4.8 yrs (range 0.2 to 11.5 yrs) without statistically significant differences between strategies.

With strategy A, 49% of children with related donors survive after BMT in CR2. By choosing strategy B, 35% can be rescued with chemotherapy alone and 65% will subsequently relapse. Assuming a CR rate of 0.50 after 2nd relapse and based on the above calculated EFS rate, another 16% of the children will survive after BMT in CR3, yielding a total cure rate of 51% for strategy B. Results were strikingly different for a smaller group of children with prognostically poor features, in whom chemotherapy failes completely.

Conclusion: In total, proportions of children surviving a first relapse are similar with both strategies. In strategy B, however, only one third of the surviving patients will have received and be at risk for late effects of BMT. In contrast, BMT in CR2 must not be withhold from children with early isolated BM relapse, very early combined BM relapse or BM relapse of T-cell ALL.

O-152

VP16 / 2-CDA THERAPEUTIC WINDOW PRE-BONE MARROW TRANSPLANT CONDITIONING REGIMEN FOR CHILDREN WITH RELAPSED OR REFRACTORY LEUKEMIAS

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We assessed the toxicity and feasibility of a 5 day drug combination: etoposide (VP16 100 mg/m²/d one hour infusion) and 2-chlorodeoxyadenosine (2-CDA 10 mg/m²/d continuous infusion) were given as a pre-bone marrow transplant (BMT) therapeutic window starting 15 days prior to BMT conditioning. Fifteen children (1-3 months from relapse or treatment failure) were treated from January 1995 to January 1996. Ten had relapsed (4 ALL, and 6 ANLL) and 5 were refractory to chemotherapy (1 ALL, 3 ANLL, and 1 CML). Median age was 8 years (range; 1-21). All 15 had received intensive chemotherapy (9 patients had prior epipodophyllotoxin). Grade 4 hematologic toxicity occurred in all patients, bacteremia occurred in 4, and disseminated fungal disease in 1. Bone marrow responses after VP16/2-CDA were: 2 complete remissions, 5 residual leukemia with marrow hypoplasia (3 of whom were refractory to prior therapy), and hypoplastic marrow without evidence of leukemia in 7. BMT conditioning commenced without awaiting hematopoietic recovery in 8 patients. Median time from start of VP16/2-CDA to conditioning was 16 days (range; 13-51). Three patients did not undergo BMT because of septic death (n=1), and progressive disease (n=2). Six patients underwent related BMT (4 haploidentical, 1 HLA matched, 1 mismatched) and 6 unrelated (3 HLA matched, 3 mismatched). Median time to neutrophil count >500/mm³ was 15 days (range; 11-28). Ten are alive in CR at a median of 128 days (range; 38-368), and 2 had recurrent disease. Pre-BMT conditioning **intensification** with VP16/2-CDA is tolerable and warrants further evaluation to assess its efficacy in improving leukemia-free survival.

O-153

COMPARISON OF ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) FOR TREATMENT OF ACUTE MYELOBLASTIC LEUKEMIA (AML) IN 41 CHILDREN IN FIRST COMPLETE REMISSION

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We give an update of our results with allogeneic and autologous unpurged BMT in 41 children with AML in 1st CR.

Methods: In order to improve the results of treatment of children with AML we transplanted from 1982 to 1993 forty one children with high risk of relapse in first remission.

	Allogeneic BMT	Autologous BMT
No.of patients	17	23
Median age (yrs./mths.)	1/09-16/03	1/06-17/06
Age range (yrs./mths.)	11/07	6/06
Months from diagnosis to BMT (median)	8	9
WBC at diagn. > 20 Gpt/l	6	14

All patients with AML received induction, consolidation and maintenance therapy according to the AML 1 (81)- or AML 2 (87)- protocol. After conditioning with cyclophosphamide and total body irradiation or with cyclophosphamide and busulfan 17 children who had HLA-identical sibling donors received allogeneic marrow transplants, 23 without a matched sibling donor received autologous unpurged marrow. Prophylaxis for graft-versus-host disease (GvHD) consisted of MTX and prednisolone, since 1989 of CyA, MTX and prednisolone. Indications for BMT in 1st remission were initial white blood cells above 20000/µl, FAB subtype M 4 - M 7, time to remission longer than 70 days and AML as a second malignancy. In contrast to most other groups our autografted children received thioguanine and MTX for one year as an equivalent for the lack of graft-versus-leukemia reaction. 15 of 41 children were examined for late pulmonary toxicity.

Results: Allogeneic BMT: Ten of 17 children remained in 1st CCR at a median follow up of 28 months. The estimated EFS for 10 years was 56 ± 12 %. Four patients died of BMT-related complications (leukencephalopathy, CMV-pneumonitis, Aspergillosis and aGvHD IV). Three children relapsed. Chronic GvHD occurred in 5 children (42 %). Autologous BMT: The overall event-free-survival rate for 23 children after ABMT was 68 ± 10 % with a median follow-up of 57 months. None of the 23 children died of BMT-related toxicities. 7 of 23 children relapsed. The only site of relapse was bone marrow. In 3 of 15 patients restrictive lung changes with a vital capacity < 70 % were observed. The forced expiratory volume in 1 second (FEV_1) was normal in 12 of 15 patients.

Conclusions: BMT, wether allogeneic or autologous, is a safe procedure in children with an acceptable risk for complications.

Both allo- and autografting are effective for maintaining remission although there is a trend toward a higher relapse rate in the autologous group. Our results strongly support our strategy to transplant children with high risk AML in 1st remission as an effective alternative therapy.

O-154

EFFICIENT AND SAFE PBSC COLLECTION IN CHILDREN

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We report our experience of 334 leukapheresis (LP), 143 series, performed in 99 children with solid tumors (60), lymphomas (13) and leukaemias (26). Body weight was 21 kg (6.8 - 75), age 8 yrs (0.5 - 18). LP were performed in pediatric environment on Cobe Spectra after sequencial or mobilizing chemotherapy +/- HGF, or HGF alone. Access/return were: 2 peripheral veins (pv) for 14.3 %, or central line (Broviack) inserted at the diagnosis with pv for 71.6 % LP. For 14.1 % LP a temporary line was inserted and used with Broviack or pv. 2 (1 - 4) LP were performed in each serie, 0.4 (0.1 - 3.5) 10^6 CD34+cells/kg/1 blood volme processed (bvp) with 10.5 (0.6 - 213.5) 10^4 CFU-GM/kg/1 bvp were obtained. There were no significant effects on blood counts except of platelet decrease (30%) following each LP. **LP in pts < 15 kg.** 105 LP were performed in 35 pts. For pts with blood volume (bv) < 1L and/or a Hb level < 120 g/L the extracorporeal line (150mL) was primed with packed red cells. ACD ratio was 1/18, Ca gluconate (0.5g/kg) was administered before and every 60 mn during LP. Access/return were: 2 pv for 3/105 (2.8 %) or Broviack with pv for 74/105 (70.5 %) LP. For only 28/105 (26.6%) LP a temporary femoral (23) or jugular (5) catheter was inserted and used with Broviack. Blood flow rates were gradually increased to the maximum achievable and ranged 7 - 20 mL/mn (median: 13mL/mn). 4.5 (1.1 - 10) bv were processed, 1.2 (0.5 - 5.3) 10^6CD34+cells/kg/1 bvp with 14.6 (4.4 - 177) 10^4 CFU-GM/kg/1 bvp were collected. Problems associated with LP in the smallest of pts can be overcome using low blood flow rates, low citrate ratio and priming the extracorporeal line if necessary. **LP after G-CSF-alone priming in pts with solid tumors and ALL.** In 30 pts with solid tumors LP were performed after G-CSF (10µg/kg/day) and daily monitoring of CD34+cell mobilization kinetics. 4.3 (2 - 7) bv were processed, 0.8 (0.3 - 4.7) 10^6 CD34+cells/kg/1 bvp with 30 (1 - 120) 10^4 CFU-GM/kg/1 bvp were collected. The same priming was used in 12 pts with ALL in first CR. 3 (1.8 - 4) bv were processed, 0.9 (0.2 - 3.8) 10^6 CD34+cells/kg/1 bvp with 12.3 (1.1 - 112) 10^4 CFU-GM /kg /1 bvp were obtained. G-CSF-alone priming is as efficient as chemotherapy +/- HGF mobilization since LP times can be precisely planned. LP may be an alternative to BM harvest in pts with ALL. **LP for CD34+cells immunoselection in pts < 20 kg.** In 14 pts with advanced neuroblastoma, immunoselection (Ceprate) of G-CSF-alone-primed PBCD34+ cells was performed using two pooled LP-products. Processing of 4.6 bv per pt was sufficient to achieve, after immunoselection, the thershold dose of 10^6 CD34+cells/kg for all pts. In 11/14 pts enough PBSCs to plan immunoselection (>2.5 10^6 /kg) was obtained with only 3 bvp. G-CSF-alone priming is convenient for immunoselection. One LP may be sufficient to performe it. **LP after BM or PBSC transplantation.** In 12 pts re-mobilization and LP were performed after autologous transplantation followed myeloablative chemotherapy and/or TBI. 6.6 (0.6 - 80.5) 10^4CFU-GM/kg/1 bvp were obtained. In heavely preatreated children efficient collection may be obtained.

O-155

STEM CELL TRANSPLANTATION - DIFFERENTIAL NK CELL ACTIVITY BETWEEN CORD BLOOD AND BONE MARROW

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Background and Aims: The ability of cord blood to repopulate bone marrow after myeloablative therapy is now well established. Much of the immune function however of such cells remains to be elucidated . Natural killer (NK) cells are acknowledged to play a pivotal role in mechanisms of graft vs host disease. The aim of this study was to determine the relative cytotoxic potential of cord blood (CB) and bone marrow (BM) derived NK cells for a variety of tumour targets.
Methods:NK cell and LAK induced killing by necrosis was examined using a ^{51}Cr release assay with K562 and Daudi target cells respectively. LAK induced killing by apoptosis was measured using an ^{125}I-UdR release assay with YAC-1 target cells.Granzyme B activity,an enzyme essential for apoptotic activity, was measured using a colorimetric substrate assay and expressed as arbitrary O.D. units.
Results:Freshly isolated CB cells had higher cytotoxic activity against K562 cells than BM cells (CB:37.4%+/-12.1 vs.BM: 29.5%+/-9.9; E:T 50:1).
Both effector cell sources killed Daudi target cells with CB having significantly higher LAK activity compared with BM cells (CB: 86.0% +/-11.6 vs BM45.7% +/-25.0; E:T 25:1, p<0.005).
CB LAK cells efficiently killed YAC-1 targets (66.1% +/-9.7, E:T 25:1), in direct contrast to BM derived cells which demonstrated no killing.
Co-culturing with IL-2+IL-12, IL-2+anti-IL-4 antibodies andIL-2+anti TGF- beta antibodies had no effect on killing of YAC-1 targets by BM cells.
Granzyme B activity was significantly greater in CB compared with BM cells (0.71+/-0.41 vs. 0.13+/-0.09, p<0.005).
Conclusion: NK and LAK activity are significantly greater in CB compared with BM, with respect to target cell killing by either necrosis or apoptosis and in the expression of Granzyme B. This higher level of NK cell function could exert greater graft vs. leukaemia effect than conventional bone marrow transplantation.

O-156

A RANDOMISED TRIAL OF TWO CHEMOTHERAPY REGIMENS IN OPERABLE OSTEOSARCOMA - AN EOI STUDY

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Previous EOI studies have demonstrated that the combination of cisplatin and doxorubicin appear to be as effective as more intensive multidrug regimen in osteosarcoma. The aim of this study was to compare, in a randomised trial, the two drug regimes with the "gold standard" Rosen T10 protocol. Between 1986 and 1992 407 patients with operable osteosarcoma were entered into the trial. 15 were ineligible. 199 were randomised to receive arm A: Cisplatin (CDDP) (100mg/m^2) x1 and Doxorubicin (25mg/m^2) x3, 3 courses before surgery at 9 weeks and 3 after. 193 received the multidrug regime, arm B, consisting of Vincristine (VCR) (1.5mg/m^2) and Methotrexate (HDMTX) (8mg/m^2) on days 0, 8, 35, 42 and Doxorubicin (DOX) (25mg/m^2) on days 15, 16, 17. Surgery was scheduled at week 7. Postoperative treatment was with BCD, Bleomycin (15mg/m^2), cyclophosphamide (600mg/m^2) and dactinomycin (0.6mg/m^2) x2, on week 9. Further cycles of VCR and HDMTX were given on weeks 12, 13, 17 and 18 and a further dose of DOX at week 14. On week 20 the regime changed to DOX (30mg/m^2) x2 and CDDP (120mg/m^2) on weeks 20, 23, 29, 32, 38 and 41 with further cycles of BCD at weeks 26, 35 and 44. The 2 drug arm was completed in 18 weeks whilst the multidrug regime lasted 44 weeks. All eligible patients are included in the analysis.

There were no major differences in serious toxicity between the two arms apart from more liver toxicity in the HDMTX arm. 106 (53%) patients in arm A had endoprosthetic replacement of their primary tumour compared to 94 (49%) in arm B. Tumour specimens were available for assessment of post chemotherapy histological response in 137 patients in A and 129 in B. 30% of patients in arm A had a good response (>90% tumour necrosis) and 29% in arm B.

Progression free survival (PFS) at 3 years was equal in the two arms at 42%. Overall survival (OS) was equal in both arms at 3 and 5 years (65% and 55%) OS and PFS were very similar in both regimens for patients who had either a good or poor histological response. Poor responders were not "rescued" by switching to CDDP/DOX.

We conclude that in a randomised setting a short, 2 drug treatment (A) is equivalent to a much longer and more expensive multidrug regimen (B). Arm A has been taken forward to the next study.

O-157

ADJUVANT CHEMOTHERAPY OF OSTEOSARCOMA: END RESULTS OF THE MULTI-INSTITUTIONAL OSTEOSARCOMA STUDY (MIOS)

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The MIOS was initiated in 1982 and designed to define the role of adjuvant chemotherapy in the treatment of non-metastatic high grade osteosarcoma of the extremity. After definitive surgical therapy of the primary tumor (amputation or limb-sparing resection) patients were randomly assigned to receive 42 weeks of intensive adjuvant chemotherapy post-operatively (Chemotherapy) or to observation with no further therapy until disease recurrence (Control group). Preliminary results of this study have been reported previously. We report here end results of the study including 12 year follow up of enrolled patients. 36 patients were randomized--18 to Chemotherapy and 18 to the Control group. An additional 77 eligible patients who declined the randomization but accepted treatment according to one of the two study regimens were followed in identical fashion; 59 elected Chemotherapy and 18 elected observation alone. Finally, 88 additional patients were entered after the randomization was closed and all were treated with Chemotherapy. At 12 years, the projected event free survival for patients randomized to the Control group is 11%, compared to 56% for patients randomized to receive Chemotherapy (2-sided logrank p<0.001). The overall survival at 12 years for patients randomized to the Control group is 61% compared to 67% for patients treated with Chemotherapy (p=0.7). When the outcomes of randomized and non-randomized patients are pooled according to treatment, the 12 year EFS for 36 patients in the Control group is 10% compared to 59% for 77 patients receiving Chemotherapy (p<0.001), and the 12 year survival is 50% for the Control group compared to 64% for the Chemotherapy group (p=0.047). Considering all 165 patients treated with chemotherapy, the 12 year EFS is 60%, and the survival is 64%. Elevation of serum LDH at diagnosis was associated with adverse outcome for patients treated with Chemotherapy. These end results confirm our preliminary conclusion that adjuvant chemotherapy has a significant favorable impact on outcome of patients with osteosarcoma and should be recommended for all such patients.

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TREATMENT OF PRIMARY, NON-METASTATIC OSTEOGENIC SARCOMA (OS) OF THE EXTREMITY WITH PRE- AND POSTOPERATIVE CHEMOTHERAPY (CT): A LONG-TERM REPORT FROM THE CHILDRENS CANCER GROUP (CCG).

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Prior to the use of CT, only 15% of patients (pts) with OS of the extremity without metastases at diagnosis could be expected to survive. The aims of the Childrens Cancer Group CCG-782 study were to: improve event-free survival (EFS) compared with the results of the previous CCG trial (CCG-741) by using the histologic response to neoadjuvant CT to determine postoperative CT; evaluate a uniform histologic grading system measuring tumor response; examine pt characteristics which might influence EFS. 268 pts were entered. Preoperative CT consisted of 4 courses of hi-dose methotrexate (HDMTX) and 1 course of bleomycin, cyclophosphamide, and dactinomycin (BCD). Good histologic responders (<5% residual viable tumor) were treated postoperatively with HDMTX, BCD, and doxorubicin (DOX); poor histologic responders were treated with BCD, DOX, and cisplatin (CDDP). The 5-yr EFS and survival (S) were 56% and 66%, respectively. 28% had a good histologic response to preoperative CT and had a 5-yr postoperative EFS and S of 87% and 91%, respectively. Those with a poor histologic response had 5-yr postoperative EFS and S of 49% and 61%, respectively. The response to neoadjuvant CT appears to be directly related to EFS and S. Long-term follow-up data will be presented. Further improvement in outcome will result from better neoadjuvant and postoperative CT and from the identification of important prognostic factors so that therapy can be optimized from the start of treatment.

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TREATMENT OF OSTEOSARCOMA (OS) WITH AN INTENSIVE CHEMOTHERAPY REGIMEN (OS 87 PROTOCOL)

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From January 1987 to May 1992, 238 OS patients (pts) have been enregisted in 27 SFOP centers. 190 pts less than 20 years with a non metastatic osteosarcoma of the limbs are evaluable for the study. 31 has metastases at diagnosis, 4 had an OS developped on a flat bone, in 6 pts the OS was a second tumor and 7 were excluded for various reasons They were treated with a preoperative chemotherapy regimen of high dose Methotrexate (HDMTX) 8-12 g/m^2 with Citrovorum Factor on days 1, 8, 15, 36, 43, 64, 71 and Adriamycin 70 mg/m^2 on days 22, 50. 157 pts (83%) have undergone limb sparing resection and amputation was performed in 30 cases ; 1 pt received a local irradiation ; the information is missing in 2 pts. 184 pts are evaluable for the histological assessment. 100 pts were good responders (GR) (less than 10 % viable cells) and received after surgery 12 additional HDMTX and 3 courses of Adriamycin ; 84 pts were bad responders (BR) and were treated with 6 courses of Vindesine 4 mg/m^2 on day 1, Ifosfamide 3 g/m^2/d on days 1,2 and Cisplatinum 100 mg/m^2 on day 3. The acute toxicity was tolerable and no toxic death was observed. Long term toxicity is being evaluated. Median follow up is 45 months. The actuarial disease free survival is 64 % at 40 months : 80 % for GR and 50 % for BR. 11 out of 42 relapses were local. Overall survival is 78 % at 60 months. In this study, the pronostic of BR pts remains worse than the one of GR in spite of the post operative addition of Ifosfamide to Cisplatinum. (Supported by the "Association pour la Recherche contre le Cancer", Villejuif, France.)

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CHEMOTHERAPY FOR OSTEOSARCOMA (OS): RESULTS OF THE COOPERATIVE OSTEOSARCOMA STUDY GROUP COSS. S.Bielack, B.Kempf-Bielack, D.Epler, N.Fuchs, G.Delling, N.Graf, H.Jürgens, O.Koch, R.Kotz, M.Salzer-Kuntschik, K.Winkler. Cooperative OS Study Group COSS; Germany, Austria, Switzerland.

COSS has conducted cooperative trials for primary, localized extremity OS since 1977. A good histological response to preoperative chemotherapy was the most important predictor of good prognosis in all neoadjuvant studies. The most efficacious protocol so far was COSS-86, which included aggressive four drug therapy (doxorubicin (DOX), high-dose-methotrexate, and cisplatinum (DDP) with ifosfamide (IFO)). A response rate of 71% and a probability of 73% to survive metastasis-free were achieved for 153 protocol patients. There was no benefit for intraarterial over intravenous DDP. Further condensation of therapy in the follow-up study COSS-91 (giving DOX not as a single agent, but with DDP or IFO) did not lead to improved results, presumably because the DOX dose had been reduced in order to allow combinations.

Apart from attempts to increase therapeutic efficacy, COSS has repeatedly addressed the problem of treatment toxicity. An attempt to use DOX and DDP only in patients not resonding to a "milder" regimen failed (study COSS-82), emphasizing the importance of an aggressive upfront approach. In order to reduce the cardiotoxicity associated with the efficacious COSS-86 protocol, short term infusions of DOX were later replaced by 48-h continuous infusions. Therapeutic efficacy of the 4-drug combination was nevertheless maintained. Next, a randomized trial was performed comparing continuous 72-h DDP with standard 5-h application. Ototoxicity was less severe after 72-h DDP, again without a negative impact on survival.

In summary, COSS has achieved high relapse-free survival rates within a multicenter group setting. Chronic toxicity could be reduced by alterations of drug application, without a negative impact on outcome.

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DETECTION OF CONTAMINATING NEUROBLASTOMA CELLS IN PERIPHERAL BLOOD STEM CELL HARVESTS BY RT-PCR FOR TYROSINE HYDROXYLASE mRNA

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Patients with advanced neuroblastoma are currently being treated by high-dose chimiotherapy followed by autologous peripheral blood stem cell (PBSC) transplantation. Because the PBSC harvests are putatively contaminated with neuroblasts, various cell selection methods are used to purge the collected hematopoietic cells from tumor cells prior to autologous infusion. In 14 children with advanced neuroblastoma, we have shown the feasibility of immunoselection and transplantation of CD34+ PBSCs (Deméocq et al., Blood 1995, 86, Suppl. 1, abst. n°1599). To detect residual neuroblastoma cells contamination, the leukapheresis and CD34-selected products were examined by immunocytological technique using UJ-13-A, GD2, and CD45 MoAbs (detection sensitivity: 1 tumor cell in 10^5 mononuclear cells). No evidence for contaminating tumor cells was found by this test. Therefore, the application of more sensitive methods allowing improved tumor cell detection is essential in order to evaluate the PBSC harvests for contaminating neuroblastoma cells. A highly sensitive detection assay for circulating neuroblasts based on the reverse transcriptase - polymerase chain reaction (RT-PCR) using the tyrosine hydroxylase (TH) mRNA as a tissue-specific target has been recently reported (Burchill et al., Int J Cancer 1994, 57, 671). This method was able to detect 1-10 neuroblastoma cells in 10^7 normal blood cells. We used a modified RT-PCR technique for TH mRNA with nested primers yielded similar detection level as has been determined in serial dilution experiments. RT-PCR analysis for TH mRNA was performed on 9 PBSC harvests from 7 patients with advanced neuroblastoma out of 14 patients included in the study of CD34+ PBSC immunoselection and transplantation feasibility. PBSC specimens were obtained before purging and cryopreserved until required for RNA extraction. All 7 children had no bone marrow disease detectable by histological analysis. Positive control for RT-PCR included cultured neuroblastoma cell line (IMR-32). Normal bone marrow and blood mononuclear cells (n=8) served as negative controls. Four children with neuroblastoma had detectable by RT-PCR tumor cells contaminating four PBSC harvests from five. The four harvests from remaining three patients were free from tumor cells. No TH RT-PCR products were identified in all of the control samples analysed. These preliminary results of RT-PCR analysis suggest that the risk for tumor cell contamination in autologous PBSC transplantation for patients with neuroblastoma is substantial. The increase in sensitivity provided by RT-PCR for TH mRNA compared to immunocytology could be useful in screening PBSC harvests for tumor cells contamination and in evaluating the purging efficacy.

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MAINTENANCE THERAPY VERSUS MEGATHERAPY AS CONSOLIDATION TREATMENT IN STAGE 4 NEUROBLASTOMA

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With the intention to improve survival for children with stage 4 neuroblastoma, two different treatment modalities were used in the German cooperative neuroblastoma study NB 90. After intensive induction treatment, patients received either megatherapy with melphalan, etoposide and carboplatin or twelve alternating cycles of melphalan/etoposide per os and cyclophosphamide per os/vincristine i.v..

We analysed the outcome of 111 children with stage 4 registered until 31.3.1994 and who either received megatherapy (43) or started maintenance treatment (68). Follow-up time is between 1.5 and 5.5 years. Median age at diagnosis was 2.9 yrs (megath. 3.1 yrs vs maint. 2.8 yrs). Time from diagnosis to BMT or start of maintenance was 9.7 mo (9.8 vs. 9.5). Distribution of risk groups at diagnosis (Am J Pediatr Hematol Oncol 14:207, 1992) was

Risk group	low	intermediate	high
all patients	10.8 %	24.3 %	64.9 %
megatherapy group	4.7 %	20.6 %	64.7 %
maintenance group	14.7 %	30.2 %	65.1 %

Life table analysis of progression free survival yields 0.30 (SE = 0.10) for the maintenance group and 0.27 (SE = 0.08) for the BMT patients. A Cox regression analysis of the facotrs treatment modality, age >/< 2 yrs, risk group and progression free survival yes/no reveals a significant impact of age only. However, the life table analysis of patients < 2 yrs gives no significant difference between BMT (n=10, PFS 0.50; SE 0.15) and maintenance patients (n=21, PFS 0.76; SE 0.10). Bone metastases at diagnosis were found in 61 children; they had cleared completely in 40 and partially in 21 at BMT/start of maintenance. 25/40 bone CR patients survive as compared to 9/21 bone PR patients.

In conclusion, no survival advantage is seen for either of the treatment arms. There are more younger and low risk group patients in the maintenance group, otherwise the treatment groups are comparable. This possible selection factor may explain the small but nonsignificant advantage in the maintenance group.

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^{131}I-MIBG DENOVO IN LOCALIZED PROGRESSIVE NEUROBLASTOMA (NBL)

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Introduction: localized neuroblastoma, especially if progressive disease necessitates some kind of therapy. Surgery, often preceded by chemotherapy is the generally accepted option. Acute toxic effects, hospital admission and late effects can hardly be avoided by this strategy. MIBG therapy can result in favourable tumour response, *sometimes making additional procedures redundant.*
Patients: among 42 patients entered the MIBG-denovo study. 10 were having localized disease at diagnosis, 4 boys, 6 girls. Three were `1 yr old at diagnosis. All had elevated minor catecholamines, only 1/10 had n-myc amplification together with 1p deletion.
Method: 2-5 courses of ^{131}J-MIBG were administered. First dose 150 mCi, subsequent doses 100 mCi. Thyroid protection was done by giving potassium jodide to the patients. The solution of MIBG in 100 ml 0,9% sodium chloride was administered i.v. in 4 hours. The interval between courses was 4 weeks.
Results: Response prior to surgery, after MIBG according to INRC was SD1, PR8, CR1. Additional surgery was performed in 7 patients. In 3 patients MIBG-therapy alone proofed to be sufficient. Four patients had chemotherapy after MIBG and surgery. Eight patients are alive 11-64 months after diagnosis. Two died, one of these with unfavourable biologic markers.
In conclusion: If therapy is necessary in localized neuroblastoma, MIBG therapy proved to be a very good therapeutic tool. By this procedure due to major responses, additional modalities can sometimes be avoided, toxicity is minimal and survival rates are promising.

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CISPLATIN MODIFIES MIBG UPTAKE IN NEUROBLASTOMA CELL LINES.

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131-I-Metaiodobenzylguanidine (131-I-MIBG), a structural analogue of noradrenaline, is selectively taken up by neural crest derived cells, and is currently used in scintigraphic visualization of neuroblastoma (NB). 131-I-MIBG at high specific activity and at high dose is also been employed in the treatment of disseminated NB with encouraging results. Recently 131-I-MIBG radiomethabolic treatment has been utilized in conjunction with cisplatin based chemotherapy with promising results (Mastrangelo R. et al, Eur J Cancer, 31A(4): 606,1995). One possible drawback of this approach can be related to the influence of cisplatin on the uptake mechanism of MIBG. In order to evaluate the possible influence of cisplatin on specific MIBG uptake mechanism, we measured 125-I-MIBG uptake in 4 NB cell lines following cisplatin exposure at concentrations varying from 50 to 500 ng/ml for 24 and 48 hr. Human NB cell lines SH-N-DZ, SK-SY5Y, KCNR and NGP were utilized. At the end of cisplatin exposure, specific uptake of MIBG was determined at 37°C following 2 hr incubation with 0.1 μM 125-I-MIBG. Specific uptake of NB cells treated with cisplatin measured as pmol/10^6 cell was compared to control untreated cells and expressed as percentage of control. At least 2 experiments were performed in triplicate for each cell line. Statistical analysis was performed utilizing the non parametric Mann-Whitney test. SK-N-DZ and NGP cell lines showed a significant increase in MIBG uptake after cisplatin treatment with the maximum effect observed following 48 hr exposure ($p<0.01$) to 400 ng/ml of cisplatin. In contrast, a decrease of MIBG uptake of approximately 35% was observed in SH-SY5Y cells treated with 500 ng/ml for 48 hr ($p<0,05$). No significant difference in MIBG uptake was detected with KCNR cells. Our results suggest a variability of MIBG uptake in different cell lines following cisplatin exposure which may reflect the heterogeneity of the tumor from which they derive. Studies are in progress to identify specific mechanisms affecting MIBG uptake in NB cells treated with chemotherapeutic agents.

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RADIATION ENHANCEMENT BY RADIONUCLIDE THERAPY & HYPERBARIC OXYGENATION.

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Neuroblastoma stage IV in children over one year of age has a bad prognosis. There are no cure rates reported in the literature for recurrent neuroblastoma stage IV, but they are considered to be nill. It is a therapeutic dilemma in treating patients with recurrent neuroblastoma stage IV. Tumour hypoxia and certain metabolic factors remain important in the efficacy on installed treatments. The application of radiation modifiers may influence the survival rate. As radionated Meta-^{131}Iodo-Benzyl-Guanidine (M-^{131}IBG) has shown to be active in the treatment of neuroblastoma, an attemp was made to improve survival of patients with recurrent neuroblastoma stage IV combining hyperbaric oxygenation and M-^{131}IBG. As a determinant of the usefulness of a treatment for relapsed patients with a stage IV neuroblastoma , the survival was used. At 28 months a survival of 12% was measured for a group of 36 patients who were treated with at least 2 treatments of M-^{131}IBG. This is compared to a cumulative probability of survival of 40% at 28 months for 36 patients treated with M-^{131}IBG and hyperbaric oxygenation. Out of 38 patients 17 are alive in this group of patients. From this study it can be concluded that "unsealed source" radiation enhancement by hyperbaric oxygen is feasible provided that a large hyperbaric chamber is available. Since all patients treated with M-^{131}IBG & HBO had recurrent stage IV neuroblastoma after conventional therapy including bone marrow transplants, these results are promising, when compared with the results of the first phase II study on the use of M-^{131}IBG but without HBO in patients with recurrent neuroblastoma stage IV.

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LACK OF ANTI-TUMOR EFFECT OF ALL-TRANS RETINOIC ACID (ATRA) IN PATIENTS WITH METASTATIC NEUROBLASTOMA : RESULTS OF A SFOP STUDY IN 13 PATIENTS.

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In order to evaluate the safety and efficacy of differentiation therapy in patients (pts) with neuroblastoma (NB), a phase II trial of intermittent oral ATRA administration was initiated. **Pts and methods**: pts were eligible if they had histologically proven NB, evaluable disease, primary refractory after 3 lines of therapy or relapsing after high-dose chemotherapy, no intra-cranial tumor, a life expectancy of 3 months and a Lansky score > 40%. Written informed consent was obtained from pt's parents. ATRA was administred orally at a dose of 30mg/m^2/12 hours for 7 days (d), followed by 7 d rest; a new course was started by d 14. Four courses of treatment were planed before disease evaluation, except in case of early clinical progression. A Fleming trial plan was designed in order to detect a 10% response rate by consecutive inclusion of 36 patients, with 2 interim analysis. **Results** : 13 pts (12 stage 4 and 1 stage 3, median age 3 years at diagnosis) were included between May and December 1995, at the time of their first (5) or subsequent relapse or progression (8). Delay between diagnosis and inclusion ranged from 8 to 75 months (med. 20). Sites of disease at entry was primary (3), bone marrow (8), bone by MIBG (12). Compliance to treatment was good and toxicity consisting of less than WHO grade 3 headache in 9/13 pts during the first week, mild cutaneous and gut toxicity was observed. One patient presented with hypertensive encephalopathy, associated with a high noradrenaline excretion 2 weeks after completion of treatment. *Progression of disease was observed in all patients, either in the primary* (1), or in pre-existing (6) or new (6) metastasic sites. **Conclusion** : the study has been closed since intermittent administration of ATRA at the recommended dose for phase II studies in children is non toxic and not effective in pts with metastatic NB.

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DISSECTING THE MOLECULAR PATHWAY THAT LEADS TO THE GENERATION OF EWING TUMORS

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The *EWS* gene is rearranged with genes encoding DNA binding proteins in several rare tumors. In Ewing tumors (ET), *EWS* is consistently fused to certain members of the *ETS* oncogene family, *FLI1*, *ERG* or *ETV1*. Although the genesis of ET is largely unknown, this gene rearrangement is considered as the primary transforming event. Three functional domains are present in all *EWS*-fusions: a DNA-binding domain, a transcriptional transactivation domain and a 82 amino acids N-terminal transforming domain. In order to unravel the pathway that leads to the generation of ET, we screened for proteins interacting with the *EWS* transforming domain by means of the yeast "two-hybrid-system". Among potentially *EWS*-interactive partners, a small nuclear protein was identified which is part of the basic transcriptional machinery. This protein also coprecipitates with the *EWS-FLI1* fusion protein in-vitro. Since the transforming activity of the N-terminal *EWS* portion has been shown to be dependent on the presence of the *FLI1* DNA-binding domain, we hypothesize that direct interaction with part of the RNA polymerase II holoenzyme may facilitate efficient recruitment of the basal transcriptional machinery to specific transforming target genes. Previous studies using degenerate oligonucleotides revealed overlapping DNA binding specificities for almost all *ETS* proteins. In order to identify *EWS-FLI1* restricted genes, we determined the spectrum of *ETS*-related transcription factors present in an ET cell line and developed an immuno-coprecipitation assay to isolate genomic DNA fragments specifically bound by *EWS-FLI*. Five additional *ETS* proteins which we found to be coexpressed with the chimeric *EWS-FLI1* in ET will have to be considered in the future specificity testing of potential *EWS-FLI1* target genes.

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FACULTATIVE ABERRATIONS IN EWING TUMORS: DO GENETIC DATA CORRELATE WITH CLINICAL PARAMETERS?

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Ewing's sarcoma, peripheral Primitive Neuroectodermal Tumor and Askin tumor, summarized as Ewing tumors (ETs), are characterized by a high MIC2 expression and the t(11;22)(q24;q12) or other rearrangements involving the EWR1 locus at 22q12. In addition to these primary aberrations, nonrandom secondary chromosomal aberrations have been reported: trisomy 8 and 12, the unbalanced translocation t(1;16) and deletions at 1p36. To evaluate the frequency and to study the biological impact of these additional aberrations, we analyzed ET specimens from 58 patients by classical cytogenetics and/or in situ hybridization (ISH) techniques. Information on the clinical outcome was available in 54 cases. The copy number of chromosomes 8 and 12 and the presence of a der(16)t(1;16) were evaluated by fluorescence in situ hybridization (FISH) with paracentromeric DNA probes. Deletions at 1p36 were analyzed by FISH with DNA probes specific for 1q12 and 1p36. In this study, the t(11;22) and other rearrangements of 22q12 were found in 88% (30/34) of the cases analyzed cytogenetically. Gains of chromosomes 8 and 12 were detected in 55% (32/58) and 24% (14/58), respectively. Loss of chromosome 16 or der(16)t(1;16) chromosomes were found in 20% (10/51), deletions at 1p36 in 16% (8/51) of the cases evaluated. Gains of chromosome 20 occurred in 21% (7/33). Within the group of tumors harboring loss of chromosome 16, a der(16)t(1;16) or a deletion at 1p36, ten of the patients showed progression of disease or died of disease, four patients are in complete remission (median follow-up 2.5 years). The presence of the aberrations was compared with the localization of the primary tumor and the extent of the disease at diagnosis by chi-square analysis and Fisher's exact test, respectively. In localized ETs analyzed in this study ($n = 22$) deletions at 1p36 were the only aberrations identifying a subgroup of patients with a statistically significant unfavorable outcome ($p < 0.02$). The relatively small number of tumors analyzed and the relatively short follow up time have to be considered.

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EXPRESSION OF DIFFERENT EWS-CHIMERIC TRANSCRIPTS AND CLINICAL RISK GROUPS OF EWING TUMOR PATIENTS

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Fusion between *EWS* and *FLI1* or *ERG*, genes in Ewing tumors (ET) is detectable in about 95% of cases by RT-PCR. In a European multicenter study, 147 ET were analysed and the molecular data were compared to all clinical data available. Most tumors expressed chimeric transcripts with fusion of *EWS* exon 7 to *FLI1* exon 6 (75/147)(type I) or 5 (39/147)(type II) and *EWS* exon 10 to *FLI1* exon 5 (8/147) or 6 (5/147). Additionally, in 5 of 147 cases rare transcripts were observed with chimerism between *EWS* exon 9 and *FLI1* exons 4 and *EWS* exon 7 and *FLI1* exon 7 or 8. Moreover, 15 cases of *EWS-ERG* rearrangement were identified.

In 85 of these patients treated in the European Cooperative Ewing Sarcoma Studies (CESS 81, CESS 86, EICESS 92) molecular results were analyzed in comparison to age, sex, tumor localization, tumor volume, and disease-extension. No significant correlation between the various fusion types and these features were observed. After a median observation time of 32 months relapse-free survival for the 31 patients with localized disease and fusion type I tended to be longer compared to the 24 patients with localized tumors bearing other chimeric transcripts ($p=0.0095$). In contrast, no significant difference in progression free survival in relation to EWS fusion type expression was observed in patients with metastatic disease. Although the time of observation is still very short, our results underline the importance of detailed fusion type characterization in the diagnostic workup of ET.

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MOLECULAR STAGING AND p53 ALTERATIONS IN EWING FAMILY TUMORS

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Ewing family of tumors (ETs) are identified by specific translocations generating chimeric transcripts - FLI/EWS, ERG/EWS, ETVI/EWS or E1AF/FWS. We studied 45 ETS: 26 for the chimeric transcripts and 35 for the involvement of the, p53 gene. FLI/EWS was identified in 69%, and ERG/EWS in 31% of the patients. The incidence of ERG is significantly higher (10%) than reported in other populations. All 4 Arab patients had the ERG/EWS transcript. To assess the degree of dissemination, bone marrows (BM) and peripheral blood (PBL) were investigated for the chimeric transcripts at diagnosis and during the course of the disease. Ten out of 13 children were found positive in BM, PBL or both, of whom 6, at diagnosis without over microscopic involvement, in 4. In some, the detection of transcripts in the BM proceeded the overt tumor progression. p53 gene alterations, LOH, SSCP, and sequencing analyses, were performed in 35 children. LOH and mutations were detected in 3 (14%) and 4 (12.5%) patients, respectively, mostly during progression. An inferior event-free survival was observed in those with LOH and or mutations ($p=0.0167$). This is the first report indicating a correlation between p53 involvement and poor outcome in ETs. Our observations suggest that BM and PBL involvement is a frequent event in ETs and should be integrated in the staging and detection of minimal residual disease. More data, and longer follow-up is required to estimate the clinical significance of our observations.

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LIMB SALVAGE FOR SKELETAL SARCOMA IN CHILDREN AND ADOLESCENTS

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From January 1981 through December 1995, 83 children and adolescents underwent subamputative surgery for either high grade (n=77) [osteosarcoma (OS) 53, Ewing's sarcoma (ES) 16, malignant fibrous histiocytoma (MFH) 4, others 4] or low grade skeletal malignancies (n=6). Primary sites included distal femur (n=21), proximal humerus (n=19), proximal tibia (n=12), proximal femur (n=10), proximal fibula (n=10), midshaft femur (n=5), others (n=6). The most common surgical procedure performed was that of enbloc resection and insertion of custom made prosthesis in 61 patients (pts). In 11 this consisted of either an expandable prosthesis or that of modular design. Intercalary grafting using bone bank allograft was performed in 5 pts. Vascularized fibular graft was used in 2 pts with distal radius lesions. In 3/11 pts with proximal fibular lesions, banked bone graft was used to support the lateral tibial condyle. Followup has ranged from 2 months - 15 years (median 40 mos). Of the 28 pts who died, 26 had functional extremities. Amputation was performed in 11 pts for infection (n=7), loosening (n=3), and local recurrence (n=1). Three pts with loosening have undergone replacement of their endoprosthesis. Intraoperative complications were primarily related to excessive blood loss (15-20 cc/kg) and three 3 pts had vascular injury, requiring repair. Postop complications included transient nerve palsy (n=8), skin necrosis (n=18), requiring debridement/complex closure (n=7). The degree of personal satisfaction with functional outcome was noted to be 80% in patient survey. In conclusion, limb salvage procedures a) are relatively safe, b) can be achieved with an acceptable morbidity, c) provide excellent local control, d) yield good patient satisfaction.

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LONG TERM RESULTS OF HIP ROTATIONPLASTY AFTER TUMOR RESECTION IN CHILDREN

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Tumors of the proximal femur and the acetabulum are difficult locations for prosthetic reconstrction especially in the children under 10. Hip rotationplasty is a well established procedure in this situation.

To date we investigated 11 patients, who underwent tumor surgery more than ten years ago to evaluate surgical complications and their social and functional rehabilitation.

Only one of these patients got surgical revision after tumor resection. The indication therefore was varus deviation of the knee joint during growth which needed a correction osteotomy.
On examination all patients showed an active hip-flexion of 70° on average (passive 90°). Four patients showed slight collateral instability in the rotated knee joint without any clinical relevance. Radiographs of all patients didn't show any osteoarthritic changes.
To prevent varus deviation, we nowadays prophylacticly perform 10 to 15 degrees valgus adjustment in the sagittal plane related to the childs age.
Three patients still attend school resp. university. The other six are working full time. Due to the excellent function each patient would choose the hip rotationplasty again for reconstruction.

The hip rotationplasty with its rotated knee provides excellent long term results. Patients functional and social needs are fully satisfied by this procedure. Prophylactic valgus adjustment at initial surgery helps to prevent later valgus deviation.

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RESULTS OF SURGICAL MANAGEMENT OF PELVIC EWING'S SARCOMA (ES). EXPERIENCE OF THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP) IN 27 PATIENTS.

Mascard E, Carrie C, Dubousset J, Missenard G, Kohler R, Oberlin O. for the committee of the study. (1) Hopital Saint Vincent de Paul, University Paris VII, France.

Ewing's sarcomas occuring in the pelvis have been recognized as to have a poorer prognosis than extremity lesions. In this study we report the outcome of 27 patients (pts) treated for non metastatic pelvic sarcoma with surgery + radiation therapy (RT) in addition to chemotherapy (CT). All pts were treated according to the protocols EW84 to EW93 of the SFOP.

Patients: the pts age ranged from 6 to 28 years with a mean of 13.3 yrs. 15 were males. Lesions involved iliac wing or sacrum. All pts underwent 'en bloc resection' of the primary after initial CT. 11 received additional RT. All received maintenance chemotherapy.

Results: Resection was complete in 24 cases, intralesional in 2 and uncertain in one. Histological response to chemotherapy was good in 20 cases and poor in 7. Probability for relapse-free survival was 67,5 % at 5 years and 52 % at 10 years. At last follow-up, mean 4.1 yrs after surgery (4 months to 9 yrs) , 18 pts had no evidence of disease, 2 had evolutive disease and 7 were died from disease. Relapses were distributed as follows: 5 local (4/5 had not received RT after surgery), 8 metastases. Functional results are being studied in this group of patients.

Conclusion: a surgical approach of pelvic ES has resulted in improvement of prognosis of this tumor. Moreover, it alloows the evaluation of histologic response to CT that will guide the post-operative treatment. RT must be associated to improve the local control in case of uncomplete resection or uncomplete response to CT.

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LOCAL RECURRENCE AFTER SURGICAL TREATMENT AND CHEMOTHERAPY

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Local recurrence in patients with localized osteosarcoma of the limbs treated by conservative surgery after chemotherapy occured in about 10% of the cases. In a population treated in France we tried to find predictive factor of local recurrence through a critical review of this large series.
We reviewed the sheets of patients treated with 2 protocols from 1986 till 1993.
The patients received preoperative chemotherapy with either high dose Methotrexate and Adriamycine or 4 drugs (Methotrexate high dose, Adriamycine, Ifosfamide and Cisplatinum). 80% of the patients had had a conservative surgery.
Among the 176 patients (pts) treated, 18 had a local recurrence, without concomitant distant metastases in 15 and with pulmonary metastases in 3 cases. We reviewed the operative and histopathological reports of pts with local recurrences. We first assessed these 18 cases. The principal features we pointed out were :
- At diagnosis : Localisation of primary tumor : femur : 14; tibia : 1; humerus : 1; fibula : 2. The Enneking surgical staging : 2 stages II_A, 15 stages II_B. The medullar involvement : 4 cases.
- At time of limb sparing surgery : the surgical management : 1 rotationplasty, 3 resections, 14 resections-reconstruction. The tumor effraction : 3 cases. The type of resection (pathological staging) : 15 wide, 1 marginal, 2 intralesional.
- The preoperative chemotherapy staging : 8 cases Huvos grading III-IV, 10 cases (55%) Huvos grading I-II.
The treatment of the local recurrence was amputation in 13 cases, local surgery in 4 cases. 8 cases received chemotherapy.
Survival after a local recurrence is 41% at 2 years with median at 16 months. In order to find some risk factors for local recurrence, we intend to match these patients with control patients, without local recurrence and similar in age, therapeutic protocol, length of follow up and histologic response (good or bad). We assessed in a logistic regression analysis, Ennecking surgical staging, surgical margins and tumoral effraction.
The results will be presented at the congress.

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USE OF ALLOGRAFT IN LIMB SAVING RESECTION FOR CHILDREN WITH MALIGNANT BONE SARCOMAS.

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Aim of study : to evaluate feasability and morbidity of bone allograft in children treated for bone sarcomas in our institution.

Methods : for the last 15 years, 45 patients underwent surgery for bone sarcomas of the limbs : 12 had Ewing sarcoma (ES) and 33 osteogenic sarcoma (OS). Orthopedic surgery consisted of amputation in 9 (20 %), endoprosthesis in 18 (40 %) and intercalary or osteoarticulary allograft in 18 (40 %). All patients received pre- and post-operative chemotherapy : combination of vincristine, actinomycin, cyclophosphamide, doxorubicin in ES and polychemotherapy in OS according to the EOI (European Osteosarcoma Intergroup) protocol.

Results : bone allograft was performed in the following primary sites of disease : pelvis (n = 3), femur (n = 11), tibia (n = 1), humerus (n = 2), ulna (n = 1). Amongst those 18 patients, only 2 died of disease 22 and 23 months following surgery. Sixteen children are alive, with a median follow-up of 62 months (range 4 to 120 months). All patients were evaluated according to MANKIN's scoring system (Clin Orthop; 174: 69, 1985) : 4/18 (22 %) were score 1 (excellent); 11/18 (61 %) score 2 (good); 2/18 (11 %) score 3 (fair) and 2/18 (11 %) score 4 (recurrence and death). Only 1 patient developed local recurrence and no patient had infectious complication. Twelve patients had two or more orthopedic operations. The major indications of the subsequent operations were : resorption of the graft or pseudarthrosis (5), fracture (2), replacement by permanent megaprosthesis (2).

Conclusions : bone allograft, when feasible, appears to be a good surgical tool in limb saving resection for bone sarcomas. Indeed, both physical integrity and good limb function may be respected. According to the criteria described by Mankin et al for functional analysis, 15 (83 %) children had a result considered to be excellent or good. However, this aspect of quality of life is obtained by numerous orthopedic operations.

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INCREASED FIBRINOLYTIC TURNOVER FOLLOWING MAJOR SURGERY IN MALIGNANT BONE TUMORS

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We have previously shown in 24 children that activated coagulation and fibrinolysis with evidence of DIC occurred during major surgery in malignant bone tumors (1). The present study was designed prospectively to evaluate fibrinolytic changes in the postoperative period in 25 additionally patients with Ewing's sarcoma or Osteosarcoma. Blood samples were obtained from peripheral veins prior to operation, 24 hours later, days 3-5, 7-9 and 14-21 respectively. D-Dimer formation (DD: Enzygnost ^{R} micro, Behring Werke Marburg, Germany), plasmin/antiplasmin complex (PAP: Enzygnost ^{R} micro), t-PA ag, PAI 1 ag and plasminogen (plasm) activity (Chromogenix, Mölndal, Sweden) were investigated serially in duplicate 3 - 6 weeks later. The results (median and median absolute deviation) are shown in the table:

days	0	1	3-5	7-9	14-21
D-D	15 (4)	37 (11)	**43 (14)**	36 (10)	38 (10)
Plasm	130 (23)	107 (16)	**93 (24)**	145 (12)	127 (6)
t-PA	8.7 (3)	9.4 (4)	**15 (5)**	12 (4)	6 (2)
PAI 1	65 (15)	85 (15)	**39 (12)**	57 (8)	63 (12)
PAP	350 (100)	422 (160)	**510 (150)**	530 (120)	392 (120)

The maximum turnover ($p < 0.001$: compared to day 0) in the fibrinolytic system with consumption of plasminogen and PAI 1 along with increased t-PA, D-D and PAP are shown 3 -5 days after major surgery. Normalisation of fibrinolytic activation occurred within three weeks. However, D-Dimer formation was still elevated in the majority of patients at hospital discharge. Compared to our previous work (1) between days 1-5 the same incidence of severe postoperative hemorrhage (25%) was noted in this follow-up study.

1.Nowak-Göttl et al. (1995) Haematologica 80: 313-319

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PROLIFERATION AND PROGNOSIS IN EWING´S SARCOMA

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It is well known that the prognosis in Ewing´s sarcoma depends upon several factors including localisation, tumor volume and stage. By contrast, little is known about the influence of cellular proliferation.
We studied the proliferative activity in 39 cases of typical Ewing´s sarcoma (ES) and 77 cases of PNET by immunohistochemical methods (ABC technique) using four monoclonal antibodies, which all yield reliable results in formalin fixed, paraffin embedded tissue: MIB 1, Ki-S1, Ki-S5, PC 10. MIB 1 is equivalent to Ki 67, Ki-S1 reacts with topoisomerases, Ki-S5 detects a formalin resistent epitop of the Ki 67 antigen, and PC 10 reacts with PCNA. In 34 cases follow-up data were available from the EICESS study (Courtesy Prof.Dr.Herbert Jürgens).
There were no significant differences in the number of positive (proliferating) cells between ES and PNET. However, significant differences were noted in the estimated probability of relapse-free survival and the 5 year recurrence-free survival rates, when the number of proliferating cells was related to the clinical course. For each antibody a threshold level could be defined which separated the tumors into two groups with different prognosis. The threshold level vor Ki-S1 was 7%. Patients with low proliferating tumors (<7%) had a median recurrence time of 11 years and 2 months, compared with 3 years and 11 months for patients with highly proliferative tumors (>7%). Analogous values were found for the other antibodies. 5 year recurrence-free survival was more than 90% for patients with low proliferating tumors, whereas it varied between 25 and 60 % in highly proliferating tumors, depending on the type of antibody studied.
We conclude from our study that proliferative activity in ES does not differ from that in PNET. However, the study of proliferation might prove useful as an additional prognostic factor.

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THE IMPORTANCE OF CHEMOTHERAPY-INDUCED NECROSIS AS PROGNOSITIC FACTOR IN LOCALIZED EWING'S SARCOMA (ES) OF THE EXTREMITIES.

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In osteosarcoma, necrosis is an established prognostic factor. In ES this correlation was little investigated. We published preliminary results in the JCO in 1993 on 68 pts, and presented the results on 108 at the 1995 ASCO meeting. Aim of this study is to confirm the prognostic value of chemotherapy-induced necrosis in ES on a large and homogenous series of pts. From 6/83 to 12/93, 118 localized ES of the extremities were treated with preoperative chemotherapy and surgery. No preoperative radiotherapy was given. Preoperative chemotherapy consisted of 3 slightly different protocols successively activated. The method to evaluate necrosis, different from the one used for osteosarcoma, included 3 grades: total necrosis, evidence of microscopic foci, and evidence of macroscopic foci. 76 pts (64%) were continuously disease free at an average follow-up of 80 months (from 24 to 152 months). At histological evaluation, 37 cases (31%) had a total necrosis, 35 (30%) microscopic foci, and 46 (39%) macroscopic foci. Only 2 pts with total necrosis relapsed (2%), in comparison to 10 (27%) with microscopic foci, and 30 (65%) with macroscopic foci. This difference was highly statistically significant at univariate analyses ($p<0.00001$). A correlation was also found with the age of the pts, those younger than 15 years had a disease free survival of 80% (47/59), in comparison with 49% (29/59) for the older ($p=0.002$). Patients with tumor volume <200 ml had a better disease free survival (67% - 69/103) in comparison to patients with larger tumors (47% - 7/15) ($p<0.05$). No other variables (sex, site of the tumor, chemotherapy protocol, type of surgery, surgical margin, post-operative radiotherapy) were related to prognosis. The multivariate analysis confirmed the prognostic value for necrosis ($p=0.0001$), for age ($p=0.025$), but not for tumor volume. In conclusion, this study states the importance of chemotherapy-induced necrosis in ES of the extremities, which should be considered in the design of new therapeutic studies.

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NEURONAL MARKERS IN RELATION TO EWS GENE FUSION TYPE IN MIC2/CD99-POSITIVE SMALL BLUE ROUND CELL TUMORS

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MIC2/CD99 positive small blue rund cell tumors referred to as the Ewing family of tumors (ET) are characterized by EWS gene rearrangements. A division into peripheral primitive neuroectodermal tumors (pPNET) and Ewing's sarcoma is still a matter of debate. We investigated 37 ET for the presence of neural markers (pNSE, mNSE, S-100, chromogranin A, synaptophysin, Leu-7, GFAP and neurofilament) and the type of EWS chimeric transcripts expressed. Positivity with pNSE was found in 14 but with mNSE in only 8 tumors. Three cases showed a positive reaction with the marker Leu7. Eight tumors were positive for S-100 protein and 2 for synaptophysin. In one tumor, reactivity with an antibody against neurofilament was focally positive. No reaction could be detected using chromogranin A or GFAP antibodies in any of the tumors. Excluding pNSE, 23 tumors showed no positivity for any neural marker. Reactivity with one neural marker was detected in 10 and with two or more neural markers in only 4 tumors. In 25 tumors fusion between EWS exon 7 and FLI-1 exon 6 (type I) and in 9 tumors between EWS exon 7 and FLI-1 exon 5 (type II) were detected. Rare chimeric transcripts were present in 3 tumors. Among cases with two or more neural markers 3/4 tumors showed other than type I fusions. In contrast only 7/23 tumors with no neural differentiation expressed other than type I chimeric transcripts. Our results indicate that when pNSE is replaced by mNSE only very few tumors display reactivity with more than one neural marker. Since in this small group type I fusion transcripts were underrepresented as compared to all others, further comparative immunohistochemical and molecular investigation is warranted to define the biological significance of these preliminary findings.

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MOBILISATION OF TUMOR CELLS DURING SURGERY IN PATIENTS WITH EWING TUMOR

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Recent studies showed that malignant cells can disseminate during surgical resection of primary tumors which might support the development of metastases. Whether preoperative systemical or local treatment has any protective effect against this mobilisation is still unknown. In the present study a close monitoring in patients with Ewing tumor was performed to detect tumor cells during open biopsy before treatment and during wide tumor resection after chemotherapy and irradiation according to the potocol of the Cooperative Ewing Sarcoma Study (EICESS) 92. From seven patients up to nine blood samples were collected from the peripheral venous blood and the operation field before, during and after surgery. The method of reverse transcriptase polymerase chain reaction (RT-PCR) was used for high sensitivity detection of tumor cells monitoring expression of tumor specific chimaeric transcripts which result from chromosomal translocation. Before operations venous blood was free of tumor cells. During biopsy and surgery tumor cells were mobilized into the operation field and the peripheral blood already before resection of the bone. Even one day after operation circulating tumor cells could be detected.

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RELAPSE PATTERN IN CHILDHOOD HEPATOBLASTOMA TREATED WITH PRE-OPERATIVE CHEMOTHERAPY (PLADO) IN THE SIOPEL 1 STUDY

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Between 1.90 and 2.94 155 patients with hepatoblastoma were entered into the SIOPEL 1 study. The aim of the study was to treat all patients with pre-operative chemotherapy PLADO (cisplatin 80mg.m IV over 24 hours, then doxorubicin 60mg.m over 48 hours) and to resect primary tumour where feasible after 4-6 courses. 134 patients had pre-operative chemotherapy, 13 had delayed surgery and, of these, 100 had complete resection of primary tumour. Only 7 pts relapsed, 6 within 2 years of diagnosis. Extent of disease at diagnosis was Grp 2, 2 pts; Grp 3, 4 pts and Grp 4, 1 pt. Four pts had lung mets. All pts showed a partial response to PLADO with complete resolution of lung metastases, and all had complete resection of primary tumour and a total of 6 courses of PLADO. Four pts had a local relapse and 3 (all of whom previously had had lung mets) relapsed in the lungs. All lung relapses occurred within 12 mo of diagnosis and all but one of local relapses within 24 mo. The only pt to have a late relapse was noted to have a rising AFP level at 24 months but the site of relapse could not be identified until 35 mo from diagnosis. All pts had further surgery. 2 pts with local relapse had no additional chemotherapy and are alive ned 2 mo and 3 yrs from relapse. 1 pt died at second surgery for local relapse, and the fourth pt was treated for local relapse with surgery and radiotherapy but had subsequent metastatic and local relapses and died. All pts with metastatic relapse are alive ned 33-35 mo post relapse, after surgery and alternative chemotherapy. These data indicate that (a) pts treated with PLADO who achieve a complete response have a low local and distant relapse rate (4%, 3% respectively) and (b) tumour recurrence, if aggressively treated, does not always imply a dismal prognosis.

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PRE-OPERATIVE CHEMOTHERAPY - CISPLATIN (PLA) AND DOXORUBICIN (DO) PLADO FOR THE TREATMENT OF HEPATOBLASTOMA (HB) AND HEPATOCELLULAR CARCINOMA (HCC) - RESULTS AFTER 2 YEARS' FOLLOW-UP.

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Between 1.90 and 2.94 91 centres in 30 countries entered 155 HB patients and 40 HCC patients into this study. The aim was to treat all patients with pre-operative PLADO (Cisplatin 80 mg/m iv over 24 hours, Doxorubicin 60 mg/m over 48 hours) and to resect primary tumour where feasible after 4-6 courses. Median age at diagnosis was 1 year (0-13 years) HB and 11 years (4-15 years) HCC. Male to female ratio was 1.6: 1 HB, 2.6: 1 HCC. Pre-treatment grouping at diagnosis based on number of sectors of liver uninvolved by tumour was Group 1. 8% HB, 3% HCC; Group 2. 36% HB, 36% HCC; Group 3. 37% HB, 25% HCC; Group 4. 19% HB, 36% HCC. 83% HB patients achieved a partial response as compared to 47% HCC patients. There have been 59 deaths, 34 HB (20 tumour, 9 surgery, 2 septicaemia, 3 other) and 25 HCC (23 tumour, 1 other, 1 septicaemia). After 2 year follow-up toxicity is less than that seen from the intergroup study, and survival at 2 years is good 79% HB, 41% HCC, representing a marked improvement over historical controls. In particular, 2-year survival is good in pts with mets at diagnosis: 65% with, 82% without. Preliminary 4-year survival is estimated at 78% HB and 33% HCC.

In conclusion, our good preliminary results have been borne out on completion of 2-year follow-up. Our next generation of studies is based on these results.

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COMPARISON OF THE ANTITUMOR ACTIVITY OF CISPATLIN, IFOSFAMIDE, ADRIABLASTIN, CARBOPLATIN AND VP16 AGAINST 3 HETEROTRANSPLANTED HEPATOBLASTOMA

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The hepatoblastoma cell suspension from 3 children were transplanted into nude mice NMRI (nu/nu) and tumor growth induced. The effectiveness of the chemotherapy (CT), together with the treatment strategies of SIOP and the HB 94 study of GPOH, were analysed. One tumor was a purely embryonal multifocal hepatoblastoma, whereas the other tumors were embryonal/fetal hepatoblastoma localised on a liver lobe. The tumorised animals were given ifosfamide (I), cisplatin (C), adriamycin(A), carboplatin and VP 16 singly in 2 blocks. Afterwards, the tumor growth rate and α- fetoprotein level- before and after CT - were recorded and compared to the control group. After the CT, the tumors were histologically tested and the mitose-rate set. Also, the proliferation activity of the tumor cells was recorded semi-quantitatively using monoclone antibodies, after application of bromdeoxyuridine into nude mice. As in the clinical course, the multifocal hepatoblastoma did not react significantly to the IPA , carboplatin and VP 16 treatment. Also the α-fetoprotein level, before and after CT, as well as the tumor histology after CT, especially the mitose- rate of the tumor cells, correlated.
With the other 2 hepatoblastoma, a good response was obtained with IPA treatment ($p< 0,02$ against the untreated control group). The reaction of the tumors using VP 16 and carpoplatin singly showed, against the control group, an insignificant reduction in the size of the tumor. The different responses of the CT were also mirrored in the α- fetoprotein level, with significance to the control group ($p<0.02$) and in the tumors' mitose rate ($p<0.03$).
To summerize: it can be seen that one in vivo model for analysis of the effectiveness of CT for HB was established that corretates with the histopathological examination and labotatory analyses. The tumor response is also comparable to the clinical patient data. The animals model is good basis for the search for further, especially new, medicines for the treatment of hepatoblastoma.

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EFFICACY AND TOXICITY OF NEOADJUVANT CHEMOTHERAPY (CT) USING ETOPOSIDE (VP16) AND CARBOPLATIN IN 20 PATIENTS (PTS) WITH INTRAOCULAR RETINOBLASTOMA (IORB).

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Given the efficacy of the combination VP-16 and Carboplatin in extraocular retinoblastoma, we tested the same CT as initial treatment of intraocular tumors.
Methods : Between 07/94 and 09/95, 20 pts, presenting IORB not treatable by conservative ophtalmological approach, were included. Their median age was 9 months (1-54). They presented bilateral (17) or unilateral (3) RB. 35 eyes (e) were treated; Reese group was I (1 e), II (1 e), III (8 e), IV (4 e) or V (21 e); retinal detachment was observed in 11 e. Two courses were delivered at 3 weeks interval and evaluation was performed 6 weeks after initiation of CT.
Results : Complete fragmentation (F) of tumors was observed in 8 e, partial F in 19 e. Complete retinal reapplication was observed in 6 e; decreasing of vitreous seeding (VS) was observed in 2 e. In 3 e, VS progressed or appeared. New e tumors were observed twice. After CT, external beam radiotherapy (EBR) could be avoided for 7 e in 6 pts. Decreased tumor size before EBR was observed in 14 e of 10 pts. Secondary enucleation was performed in 5 pts showing no or minimal extraretinal extension.
Toxicity of CT was registered for 40 courses. No toxic death occured. Transfusion of platelets were necessary in 3 courses and of erythrocytes in 7. Grade IV neutropenia was observed in 23 courses with a median duration of 10 d. Hospitalization for infectious problem was necessary in 9 courses.
Conclusions :
- This drug combination is highly effective in IORB
- The toxicity is acceptable
- The benefit is clear for posterior pole tumors which become accessible to local treatments avoiding EBR
- Visual benefit for bilateral V group needs to be proven
- The risk of second tumor might be potensialized with this treatment in heritable retinoblastoma.

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CHEMOTHERAPY WITH ETOPOSIDE AND CARBOPLATIN IN INTRAOCULAR RETINOBLASTOMA

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Background: The association of carboplatin (CBDCA) and etoposide (E) has been found to be highly effective in the treatment of extraocular retinoblastoma (RB).
Objective: In order to reduce retinic scarring after argon laser therapy for low-stage intraocular lesions, and to avoid radiotherapy (RT), (external or plaque), or enucleation (en) for advanced intraocular illness, we tested the efficacy of CBDCA-E association in intraocular RB. This same chemotherapy (CHT) has also been used for high risk unilateral enucleated RB.
Patients and methods: Since 1990, 19 pts affected with intraocular RB have been treated with CBDCA-E association. CHT consisted of CBDCA (1,000 mg/sqm) and E (300 mg/sqm) on day 1, 21-28 days apart, and reduced by 1/3 in pts weighing less than 10 kg. In four pts a chromosome 13 abnormality was detected. Sixteen pts were treated at onset and three were pretreated.
Responses: Six pts (unilateral RB) received 4 courses of CHT after enucleation because of risk factors. None relapsed after a mean of 20 months after diagnosis. Thirteen pts received chemotherapy immediately followed by argon laser therapy:

	Pt	CHT	Laser	Pre-T.	Response	Relapse	Outcome/mos
Uni/n-en	1	3	+	no	CR	no	ANED/ 7
	2	3	+	no	CR	no	ANED/ 7
Bil/n-en	3	6	+	no	PD	-	DOD
	4	3	+	no	CR	no	ANED/ 6
Bil/en	5	6	+	+	PD	-	Lost to follow-up
	6	6	+	no	CR	no	ANED/ 10
	7	4	+	+	PD	-	DOD
	8	3	+	no	CR	-	Lost to follow-up/ 25
	9	7	+	no	MR	+	ANED/ 12
	10	4	+	+	PR	+	ANED/ 60
	11	4	+	no	PD	-	ANED/24
	12	7	+	no	CR	no	ANED/62
	13	2	+	no	PR	no	ANED/2

CHT was well tolerated; myelosuppression was manageable. After 2-4 courses of CHT, followed by argon laser therapy, 6 CRs, 2 PRs, 1 MR, 4 PDs were achieved. Major responses occurred in 61.5%. Two pts (1 MR, 1 PR) relapsed and underwent RT and/or enucleation; at present, they are all alive without disease. Four pts had PD: two died of extraocular disease, one was lost to follow-up, and the last underwent enucleation. Six pts did not undergo either RT (external or plaque) or enucleation; the shrinkage of intraocular lesions following CHT permitted laser therapy on smaller foci, thus reducing retinic scarring.
Conclusion: CBDCA-E is effective in reducing the mass of intraocular RB and in permitting local treatment. However, further evaluation of this approach is needed.
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CARCINOMA OF THE NASOPHARYNX IN CHILDREN, RESULTS OF A RETROSPECTIVE STUDY (1982-1993)

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For Malignant Tumors Rare Italian Study

Introduction. carcinoma of the nasopharynx (CN) is a rare tumor in childhood, infact represents less than 1% of pediatric malignancies but comprises one-third of the carcinoma of childhood. To evaluate frequency, clinical features, adequate therapeutic approach and prognosis a retrospective study was carried out by Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP).
Patient Population and results. Out of 164 rare malignant tumors from 1982 to 1993 in 18 Italian Centers, 35 cases (21.3 %), 26 indifferentiated (74.4%), 8 differenziated (22.8%) and 1 spinocellulare (2.8%). The patients (pts), 27 boys and 8 girls (M/F=3.3), aged 7 - 16 years (median 11.7 yrs) 5 had a range age 0-4 yr, 6 5-9 yr and 24 10-16 yr. The most common presenting features at onset were rapresented by mass (26 pts: 74.3 %), dyspnea +/- adenopaty (3 pts: 8.7 %), pain (4 pts: 11.4 %), cough (1 pts: 2.8 %) and 1 pts fortuitous (2.8 %). 32 pts underwent to chemotherapy (CT) in 27 associated to radiotherapy (RT), for 3 pts RT was only treatment. The association CT more frequently used was EX+ADB+VCR (24 pts), BLEO+5-FU+CDDP+MTX (1 pts), VP-16+CDDP (1 pts), EX+ADB+VCR+ARA-C (1 pts), prot.AIEOP RMS '79 (1 pts) and in 2 cases NN. 29 pts had a complete remission (CR), 22 (62.8%) are alive in I CR with a median of follow up of 25 months (range 2-126) e 7 (24.1%) showed local relapse and died. 6 pts with progression disease died to 6, 10, 20, 24, 42 and 56 months of diagnosis.
Conclusion. The results of study are in good agreement with those precedingly reported (50-70 % overall survival). Considering the difficulties due to the surgical treatment, the combined chemio-radiotherapy appears to be the first choise therapy.

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HIGH DOSE RATE BRACHYTHERAPY IN PEDIATRIC ONCOLOGY

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Purpose: The possibility to deliver tumoricidal dose to limited volume preserving normal tissue has increased the use of Brachytherapy in pediatric oncology. The introduction of High Dose Rate (HDR) in clinical practice makes this type of brachytherapy the logical option for treatment of childhood malignancies. This report analizes the experience of A.C.Camargo Hospital with HDR in pediatric oncology.
Material and Methods: From september/92 to september/95, twelve children with malignant neoplasms were treated with HDR brachytherapy techniques. The age ranged from 1 to 17 years old (5 male and 7 females). Nine children presented nasopharyngeal primaries (Lymphoepithelioma 7, rhabdomyosarcoma 2), two uro-genital rhabdomyosarcomas (vagina 1, urethra 1) and one soft tissue sarcoma of the leg (Sinoviosarcoma). Nasopharyngeal tumors were treated with metalic catheters via nasal. For uro-genital sarcomas individual aplicators were made. Interstitial brachytherapy with plastic catheters inserted during surgical ressection was used to the lesion of the leg.
Results: With a median follow-up of twelve months, 9/12 children (75%) are alive with local control of the disease and low incidence of side effects. Three patients are dead but only one with local failure. The ultimate local control was 11/12 patients (91.6%).
Conclusions: The data show that HDR brachytherapy is effective on treatment of childhood neoplasms and a useful option for localized and accessible tumors. In spite of the small number of cases the use of HDR brachytherapy in pediatric oncology is promise with high local control rate, operational and radiological protection advantages.

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THE INCIDENCE OF PAEDIATRIC DESMOID TUMOURS AND ASSOCIATION WITH FAMILIAL ADENOMATOUS POLYPOSIS.

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Familial adenomatous polyposis (FAP) is an autosomal dominant condition comprising colonic polyps and tumours of ectodermal and mesodermal origin. Although rare in the general population, desmoid tumours (DT) have been reported in 8-15% of adult patients with FAP. This association is less well understood in the paediatric population and there are no clear data to determine whether the clinical picture or management is adversely affected by the association.

We reviewed the incidence of DT and its association with FAP from the West Midlands Regional Children's Tumour Research Group's register. From 1957 to 1995, 13 cases of DT were identified in this population based register (incidence=0.3 cases/million aged under 15/year). Of these, 3 (23%) had proven or convincing clinical evidence for FAP (2 families had a mutation in the FAP gene and a first degree relative to the propositus with colonic polyps; 1 boy had clinical features of Gardner's syndrome). There was no difference in the age at presentation or pattern of disease between those children with or without the FAP association. Nevertheless, the 3 patients with FAP were the only patients who had progressive uncontrolled disease requiring continuing treatment. This raises the possibility that the association with FAP may represent a more aggressive biological variant.

Further enquiries over a 20 year period about the national incidence of these diagnoses in children (with the assistance of Mr Charles Stiller at the National Registry of Childhood Tumours) revealed only 5 other cases. Based on the West Midlands incidence we would have expected 60 cases over this period of which 15 would have had the FAP association. This raises important considerations for counselling and screening the families of all children with DT.

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CYTOKINE PROFILE IN CHILDREN WITH HODGKIN'S DISEASE

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Defective cell-mediated immunity in untreated Hodgkin's Disease (HD) has long been recognised. The nature of the impairment in HD remains enigmatic, although evidence points to immunosuppressive factors in serum or plasma of these patients. Cytokine profile, primarily serum interleukin (IL)-2, soluble IL-2 receptor (sIL-2R), IL-4 and tumor necrosis factor alpha (TNF-α) were determined in 50 biopsy-proven HD patients and in 15 age matched healthy controls. A sandwich enzyme immunoassay was used for cytokine and receptor measurements. The study group consisted of 37 boys and 13 girls with an age range between 3 - 17 years (median 8 years). In all patients the diagnosis was based on histological findings, the nodular sclerosis subtype found in 6 patients, mixed cellularity in 42 patients, lymphocyte predominance in 2 patients. Of 50 patients, 8 were in stages I and II, 12 were in stage III and 30 were in stage IV according to Rye classification. Serum sIL-2R levels were found to be significantly high in HD patients and correlated with disease stage as compared to the remission group and normal children ($p<0.01$). The number of patients with detectable serum IL-2 levels was significantly higher in patients with HD as compared to the control group. IL-4 was undetectable in all patients. TNF-α levels were found to be high in patients with advanced disease stage and B symptoms, whereas serum TNF-α levels in patients with early stage of disease were not significantly different from those of normal subjects. The role of these circulating cytokines in the clinical presentation of HD remains unclear. Our data indicate that serum IL-2 is detectable in a subset of patients, however, in a recent study IL-2 mRNA was found to be undetectable in HD tissues. Conceivably, IL-2 detected in patients with HD could actually be produced outside of the tumoral tissues and be trapped by the high levels of circulating sIL-2R in these patients. Since sIL-2R is capable of binding IL-2, it may have an imunoregulatory role by competing cellular IL-2R for the ligand and thus down-regulating the immune response. In this regard, the sIL-2R which, by binding to the host's growth factors, may inhibit the normal immune response attempting to eliminate those tumor cells. Understanding the biological significance of circulating cytokines and/or receptors will render to clinical applications of cytokine directed therapy, representing a new perspective for the treatment of certain neoplastic diseases including pediatric lymphoid malignancies.
This study has been supported by Turkish Scientific Research Council (TÜBİTAK).

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IMMUNOMODULATION INDUCED BY rIL2 IN A TREATMENT PROGRAM INCLUDING rIL2 PLUS rIL2-ACTIVATED PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FOR CHILDHOOD OSTEOGENIC SARCOMA.

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In 1991 a multimodal treatment program of chemotherapy (CT), surgery (S) and immunotherapy was initiated for childhood osteogenic sarcoma. The program started with a first cycle of continuous rIL2 i.v. infusion ($9x10^6IU/m^2$/daily x 4) followed by PBMC apheresis. The PBMC were activated in vitro with rIL2 (18.000 IU x 10^6 PBMC for 1 hr and then stored at -192°C). After pre-operative CT (VCR+HDMTX, CDDP+IFO x 2 mos) a second cycle of rIL2 plus half of criopreserved PBMC were given. Post-operative CT depended on the histopathologic response (necrosis ≥90%: 1 cycle as above; <90%: ADM x 5 mos). The remaining PBMC were given with the third rIL2 cycle, followed by a fourth cycle of rIL2 that concluded the treatment program. PBMC immunophenotype and LAK/NK activity were evaluated in 10/31 consecutive patients accrued in this program. The immunophenotype was studied with a broad panel of MoAbs including CD2,3,16,25,38,56,57. The LAK/NK activity of PBMC was evaluated with ^{51}Cr release assay with Daudi and K562 lines as targets at different target:effector ratios. These analyses were performed in vivo before and after each cycle of rIL2, and on the leucapheresed PBMC before and after in vitro activation. The main findings were: *a*) the LAK/NK activity was evocable after all rIL2 cycles, even if more striking after the first one; *b*) the LAK/NK activity of the in vitro activated PBMC was 3 to 7 fold (mean 6) higher than that of the in vivo PBMC after the first rIL2 cycle; *c*) the percent of circulating cells with NK immuno-phenotype increased in a similar pattern after each rIL2 cycle; *d*) the increase of both NK cells and LAK/NK activity was transient and returned to basal levels between the cycles of rIL2. These findings suggest that in these series CT produced an uninfluent functional depression of the LAK/NK activity. The clinical follow-up is ongoing (up to date it ranges from 8 to 24 months, median 14 months). A longer follow-up is needed to evaluate whether the magnitude of the rIL2-induced immunologic modification relates to the clinical outcome.
Supported by AIRC and Associazione Bianca Garavaglia, Busto Arsizio, Varese.

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BONE LOCALIZATIONS OF HEMATOLOGIC MALIGNANCIES. DIAGNOSTIC PITFALLS.

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From 1991 to 95, 16 pts, 4 mth-15 yr, (Me = 9 yr), 9 M/7F had bone localizations of hematologic malignancies as first manifestation (7 ALL, 3 AML, 6 NHL).12 localizations were at the lower extremity (tibia 6, femur 5, fibula 1), 8 at the upper (cubitus 3, scapula 2, humerus 2, radius 1). Vertebra were concerned and ribs 3 times, whereas skull involvement was noted in 2/3 AML and 1/6 NHL. 6/16 children had monostotic disease thus making the diagnosis more difficult. In 5/16 cases (30 %), an initial diagnosis of osteomyelitis had led to antibiotic treatment (2-12 weeks). Morphologic diagnosis pitfalls before cell phenotyping were : a 5 mth old girl with AML (M6 variant) mastoidal, sphenoidal, scapular involvement first diagnosed as Langerhans cell histiocytosis, 2 girls (12 y, 14 y) with Pre-B ALL (Mic 2+, CD20-, CD34-), first diagnosed as NHL before the presence of Calla expression was assessed on frozen specimen and bone marrow aspirate documented a > 30 % blastic infiltration. One 8 yr old boy with tibial lesion (CD45RB-, CD20-, CD3-, CD45RO-) at immunohistochemistry was considered as Ewing's Sarcoma until lack of t(11;22) and bone marrow flow cytometry (CD10+, CD19+, CD24+, CD34+) led to the correct diagnosis of pre-B, Calla+ ALL. It is worth emphasizing for the pathologist that a SRBC bone tumor with negative lymphoid markers (CD45RB, CD3, CD20) and clear Mic-2 positivity is not always an Ewing's sarcoma and can correspond to a pre-B Calla + ALL. As most B-cell markers and especially CD20 are negative in pre-B ALL, CD34 and C10 positivity, absent in Ewing's sarcoma, is important to assess. Out of 6 Pre-B Calla+ ALL, flow cytometry immunophenotyping was in 3 cases the gold standard to assess diagnosis. A recent monoclonal antibody intended for identification of the B cell lineage (CD79a, Clone HM57, Dakopatts, Denmark) was retrospectively tested on formalin-fixed, paraffin embedded material from CD20-, CD34-, Mic2+, Pre-B ALL. After microwave treatment, tumor cells were expressing this polypeptide, which appears at the pre-B cell stage and persists until the plasma cell stage. Although CD20 is an excellent marker for B cells, it's negativity in pre-B lineage lymphoblastic proliferation dictactes the use of CD79 on paraffin embedded material, CD10, 19, 21, 22 on frozen section and/or flow cytometry bone marrow immunophenotyping.

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION IN CHILDREN

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Autologous transplantation with PBSC is an effective means of treating malignancies in adults but is less well described in pediatrics. Over a 3 1/2 year period 30 patients ≤ 16 yr. (median 5.4, 1.4-16.4) with neuroblastoma(14), rhabdomyosarcoma(4), Wilm's(2), ALL(2), medulloblastoma(2), NHL(2), hepatoblastoma(1), Ewing's(1), AML(1), and Hodgkin's(1) were transplanted at Children's Memorial Hospital. A mean of 4.3 phereses were done on either a Cobe Spectra or CS-3000 (Fenwal). Mean weight was 26.2 kg. (median 18.95, 10.0-61.0). 29/30 patients were harvested through a double lumen central line(DLCVL) ranging from 6.6 to 9.0 Fr. One patient was done through a 4 Fr. peripheral central venous catheter. The mean absolute $CD34^+$ cells infused was 29.41 x 10^6 (range 1.44-225.0 x 10^6). There were 5 different ablative regimens: Thiotepa/Cytoxan(15), Thiotepa/Cytoxan /Carboplatin(9), TBI/Cytoxan/VP-16(4), TBI/Melphalan (1), Busulfan/Cytoxan/VP-16(1). All patients engrafted. Mean time to ANC >500: 15.4 days (median 14, range 9-44) and to platelets >20,000: 30.2 days (median 20.5, range 9-93). Two patients failed to achieve a platelet count >20,000: one died at day 91 in CR of multisystem organ failure, one developed ITP post-BMT but was transfusion independent after day 105. Hospital discharge occurred after a mean of 26.6 days (median 22.5, 18-64). Overall survival was 62%; PFS was 50% with a median follow-up of 346 days (61-1273). PBSC harvesting and transplantation through a DLCVL in young children is feasible, yielding a stable graft with low morbidity and mortality.

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ADDING IFOSFAMIDE (I) AND ETOPOSIDE (E) TO VINCRISTINE (V), CYCLOPHOSPHAMIDE (C) ADRIAMYCIN (Ad) AND ACTINOMYCIN (A) IMPROVES OUTCOME IN NON-METASTATIC EWING'S (EWS) AND PNET: UPDATE OF CCG/POG STUDY.

H Grier, M Krailo, N Tarbell, M Link, C Fryer, D Pritchard, M Gebhardt, P Dickman, E Pearlman, P Meyers, S Moore, S Donaldson, A Rausen, T Vietti, J Miser for POG and CCG

We conducted a trial to assess the effect of adding I/E to VAdCA for patients with EWS or PNET of bone. Patients (pts) were randomized to receive either VAdCA alone or VAdCA alternating with I/E. Courses were given every three weeks for a year. VAdCA consisted of (doses in mg/M^2) V 2 (max dose 2), C 1200, Ad 75 to cumulative dose 375 after which A substituted at 1.25. I/E courses were I 1800/d (day) x 5d and E 100/d x 5d. Treatment of the primary tumor occured at week 9 and consisted of either complete surgical resection, radiotherapy, or both radiotherapy and surgery. We treated 395 patients [198 on VAdCA, 197 VAdCA + I/E] from 11/88 to 12/92; median age was 12 yrs (range 0-28). Sites were 186 extremity, 92 pelvic and 117 other. Treatment arms did not differ by tumor size, location or histology. Adverse prognostic factors for the study as a whole include large tumors, pelvic primary site, and older age. There were 83 failures on VAdCA and 56 failures on VAdCA + I/E. There were 13 toxic deaths (4 VAdCA, 9 VAdCA + I/E). With 3 yrs median follow up of the survivors, event free survival at 4 yrs is 51% for the VAdCA arm and 65% for the patients receiving VAdCA + I/E (P=0.0005). The improvement is most notable for patients with large tumors. We conclude that the addition of I/E to standard therapy improves outcome in non-metastatic EWS and PNET.

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PROGNOSTIC FACTORS IN LOCALIZED EWING'S SARCOMA. AN UPDATE OF THE EW88 STUDY OF THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY.

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From January 1988 to January 1992, 142 untreated patients (pts) entered the EW88 protocol based on the chemotherapy (CT) used in St Jude Hospital from 1978 to 1985 (JCO:7, 208-213, 1989) and local therapy including surgery whenever possible.

Induction CT consisted in cyclophosphamide 150 mg/m2 p.o. x 7 days followed by doxorubicin 35 mg/m2 IV on day 8 for 5 courses beginning days 1, 15, 29, 50 and 71. 107 (75 %) underwent surgical resection of their tumor either at diagnosis (6) or after induction CT (101). Radiation dose was based on the quality of surgery and the extent of histological response to CT: 48 pts (%) had no radiation, 22 received reduced doses after surgery (35 to 40 grays), the other received classical high doses (≥ 45 grays).

In February 1996, median follow-up of the cohort was 55 months (5 - 94m). Median follow-up of the survivors is 66 months. The actuarial 5 year survival was 66 %. Four pts had no initial control of their tumor. relapses occurred: 25 local (± distant), 30 isolated metastases. The 5 year actuarial EFS was 58%.

Two major prognostic factors were identified: initial tumor size (± 8 cm) and response to chemotherapy (clinical good response to CT was defined as ≥ 50 % regression of the tumor. Histological response was graduated: 1. good response: ≤ 5 % residual cells; 2. intermediate response: between 5 to 30 % cells; 3. poor response: ≥ 30 % cells). These 2 factors define 3 groups of pts. Out of the 120 pts who could be evaluated: (1) 23 were poor responders to CT and had a 13 % DFS; (2) 73 pts had either a good histological response to CT or a small tumor which was not resected but showed good clinical response to CT. There EFS was 75 %; (3) the 22 pts other pts had an intermediate survival of 43 %. There was no other prognostic factors such as age at diagnosis, sex or site of the tumor. These prognostic factors are the bases of the ungoing study of the SFOP.

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PROGNOSTIC FACTORS IN EWING'S TUMOUR - AN ANALYSIS OF 975 PATIENTS IN THE GPOH/CESS AND UKCCSG/MRC STUDIES.

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A retrospective analysis was performed on a combined GPOH/CESS and UKCCSG/MRC data set of 975 patients with Ewing's sarcoma of bone registered prior to the current collaborative EICESS trial. The aim of this analysis is to further elaborate on prognostic factors and to document changes over the study period (1977-1993) in particular improvements in relapse free survival (RFS) and trends in local therapy for Ewing's sarcoma. The initial CESS-81 and ET-1 studies used a 4 drug regimen based on vincristine, adriamycin, cyclophosphamide, and actionmycin-D (VACA). In the subsequent studies CESS-86 and ET-2 ifosfamide replaced cyclophosphamide which was used in conjunction with the other 3 standard drugs (VAIA). There were other changes over the period including those in local therapy. Radiotherapy or amputation were more common in the period prior to 1986, endoprosthetic surgery was widely utilised in the later period. The key prognostic factor is the presence or otherwise of metastases at diagnosis (5 year RFS 25.3% vs. 55.2%, $p < 0.0001$). For patients with metastases there was a trend for better survival for those with lung involvement compared to those with bone metastases, or a combination of lung and bone metastases ($p < 0.0001$). In the group of patients with no metastases at diagnosis multivariate analysis demonstrated that site, age-group, and period of diagnosis had significant influence on RFS (all $p < 0.005$). Those with axial primaries had a less favourable outcome compared to other sites (5 year RFS 49.6% vs. 61.4%). Patients aged 15 or under had a superior outcome compared to older patients (5 year RFS 59.9% vs. 48.4%). For this combined data set the RFS was superior in the period after 1985 for non-metastatic patients (45.3% vs. 60.1%, $p <0.0001$) and for metastatic patients (15.7% vs. 29.8%, $p=0.016$). In addition those patients who relapsed within 2 years of diagnosis had a less favourable prognosis than for those relapsing later (5 year survival following relapse 3.9% vs. 23.3%, $p < 0.0001$).

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ITALIAN COOPERATIVE STUDY FOR TREATMENT OF LOCALIZED EWING'S SARCOMA OF BONE.

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The main goal of this study is to improve the overall survival (SUR) and the event free survival (EFS) of patients (pts) with localized Ewing's sarcoma of bone. The patients are treated with multimodal protocol caracterized by: a) high dose chemotherapy(CT) and hyperfractionated and accelerated radiation therapy(RT); b) addition of Ifosfamide(IFO) and Etoposide(VP-16) to standard chemotherapy with: Vincristine(VCR), Actinomycin-D(Act-D), Adriamycin(ADR), Cyclophosphamide(CPM).

MATHERIALS AND METHODS. Protocol outline. Pts ≤ 3O yr of age with newly diagnosed non metastatic Ewing's sarcoma or PNET of bone are elegible. Induction CT 3 courses, every 3 wk: two courses of VAdrC (VCR + ADR + CPM) alternating with one course of VAI (VCR + Act-D + IFO). Local treatment: RT is performed only if radical tumor resection is not feasible or incomplete tumor resection occurs; a total dose of 6080 cGy in hyperfractionated and accelerated modality is given. Maintenance CT: Phase 1: 5 courses, every 3 wk, VAdrC alternating with VAI. Phase 2: 5 courses, every 3 wk, VP-16 + IFO alternating with VCR + Act-D + CPM. Treatment duration: 36 wk.

Patients. Between nov.'91 and feb.'96 115 pts were enrolled in this study. 5 pts are too early; 110 pts result evaluable: 76 males and 34 females; 55 pts were ≤ 14 yr at diagnosis. Primary sites of tumor were: extremities in 66 pts; pelvis in 23, other sites in 21.

Local treatment. 57/110 pts (51.8%) had surgery alone (with limb amputation in 5); 27 (24.6%) had RT alone; 26 (23.6%) had surgery and RT. Regarding pts ≤ 14 yr, 37/55 (67.3%) pts had surgery alone (with limb amputation in 3); 8 (14.5%) had RT alone; 10 (18.2%) had surgery and RT.

RESULTS. As of 15th feb.'96, with a median follow-up of 28 months (range 2-52), 92/110 pts remained event-free. 1 pt presented local progression of disease and successively died; 1 pt had only partial response after local treatment with RT but afterwards died on therapy because of intestinal occlusion; 16 pts (6 ≤ 14 yr) relapsed: 10 pts (5 died) with metastatic disease, 1 pt died with local recurrence (temporal bone) and 5 pts (4 died) with local recurrence plus metastases.

The 4 yrs EFS rate is 76,6% (CI 95%: 66-87). The 4 yrs SUR is 85,1% (CI 95%: 77-93).The EFS compared for treatment show: surgery (76,5%) vs RT (80,8%) vs surgery+RT (68,5%); compared for tumor site: pelvis(82,6%) vs other sites(75,1%); compared for age at diagnosis: ≤ 14 yr (83,4%) vs >14 yr (69,7%).

CONCLUSIONS. The EFS and SUR rates can be considered satisfactory. Of more concern is the restricted indication to RT, especially in paediatric pts. The comparative study of the EFS by treatment and by tumor site doesn't show statistical significance; on the other hand the EFS compared by age at diagnosis show better results for patients ≤ 14 yr .

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PREOPERATIVE SIMULTANEOUS RADIOCHEMOTHERAPY IN THE TREATMENT OF EWING'S SARCOMA

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Purpose: In the european study EICESS 92 preoperative radiochemotherapy was invented in the treatment of locally advanced Ewing's sarcomas. The feasability of this modality and the local and systemic control are examined and compared with the standard local treatment (operation or radiotherapy alone or postoperative irradiation).

Patients and Methods: From April 1991 to July 1995 83 patients with Ewing's sarcoma received simultaneous preoperative radiochemotherapy. The dose was either 44,8 Gy or 54,4 Gy depending on the expected margins of resection. Usually, radiotherapy was given as a hyperfractionated accelerated split course regime. 43 patients in EICESS received radiotherapy, 41 patients operation alone, 34 patients received postoperative irradiation. Chemotherapy consisted of either VAIA or EVAIA. In a subgroup of 52 patients treated with preoperative irradiation at the University of Münster, the complication rate was examined. The median follow up of the study is 21 months.

Results: Preoperative radiotherapy as outlined in the protocol could be performed in nearly all elected patients, the operative and perioperative morbidity was not increased. Postoperative chemotherapy could be started without delay after a median intervall of 17 days. After preoperative irradiation, 16 patients relapsed including 1 local relapse. After radiation alone 15 patients relapsed including 6 local relapses. Of the purely operated patients 4 relapsed with 1 local failure; after postoperative irradiation there were 7 relapses including 1 local failure.

Conclusion: The preliminary results show that preoperative radiochemotherapy is a well tolerated treatment modality. The local control rate is good, especially considering the usually large primaries. Up to now, no reduction of systemic relapses could be observed.

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INFLUENCE OF LOCAL TREATMENT MODALITIES ON THE RELAPSE PATTERN OF PATIENTS WITH EWING TUMORS OF THE CHEST WALL (ASKIN TUMORS)

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Purpose: The intensification of both local surgery and radiotherapy has proved to reduce the risk for local relapse in children with Ewing Tumors. Askin Tumors, however, present a particular challenge for local therapy due to limited possibilities for radical surgery and due to the neighbourhood of radiosensitive risk organs such as heart, lung and spinal cord. We examined in how far central radiotherapy planning and advanced radiation techniques improve local tumor control. Further, we analysed individual therapy modalities in patients with local therapy failure to find out factors that might have contributed to the occurence of local relapses.

Patients and Methods: Since 1981 147 patients with Askin tumors have been treated according to the study protocols CESS 81, CESS 86 and EICESS 92. 110 of them, who received as local therapy either irradiation alone or surgery and postoperative irradiation , are considered further. The cumulative risks for local vs. systemic relapse were compared, the influence of tumor factors (initial tumor burden, histological subtype) on the incidence of relapse were examined. From all patients with local (n=11) or combined local and systemic (n=5) relapse the CT images of the initial and the recurrent tumor were analysed and compared with the modalities of local treatment applied.

Results: The probability for event free survival of all studies is 0,50 (S.E. 0,02), which could be slightly improved from 0,43 in CESS 81 to 0,57 in EICESS 92. While the rate for systemic relapse did not change significantly (0,33 in CESS 81, 0,40 in the following studies), the rate for local relapse could be reduced from 0,36 to 0,16. Other factors influencing the probability for local relapse are the initial tumor volume and the histological subtype. There was no difference in local relapse rate between patients who received surgery plus irradiation and patients with definitive radiotherapy.

Conclusion: Based on the poor local control obtained in the CESS 81 Study a central therapy planning center was established for the trial, the radiation portals have been planned according to radiographic images and the doses for postoperative and definitive irradiation have been increased. Our results confirm that in Askin Tumors this stragegy has been successful. Prognostic factors, such as tumor volume and histological subtype, might help to further intensify therapy in risk patients.

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NON METASTATIC EWING'S SARCOMA FAMILY OF TUMORS (EwFT) OF THE RIBS: EXPERIENCE OF THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP) IN 55 PATIENTS

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Management of EwFT of the ribs have changed with the introduction of initial chemotherapy (CT) that allowed the use of surgery in a combined modality approach. 55 patients (pts) presenting with non metastatic rib EwFT were treated according to the protocols SFOP-EW78 to EW93.

Patients: The pt age ranged from 2 to 32 years (with a median of 11.8 yrs). 70 % of the tumors were large (> 8 cm). Pleural effusion was present in 26 pts (47 %), analyzed in 14 and containing tumoral cells in 8/14 (57 %). 7 pts had epidural involvement. For local therapy 4 pts underwent radiation therapy (RT) alone, 16 surgery alone (S) and 35 a combination of both S and RT.

Results: As of february 96, 37 pts are alive with a 77 month median follow-up. The 6 yr overall and disease-free survivals are respectively 65 % and 59 %. Two pts could not achieve first remssion and died. Relapses were distributed as follows: 6 local or loco-regional (LR) , 4 local + systemic, 11 systemic. None of the 4 pts treated without sugery are alive. 12 / 35 pts treated with RT combined with S relapsed (locally ± metas 5, meta 7), 7/16 pts treated with S without RT relapsed (local ± metas 3, meta 4).

Concerning the subgroup of 25 patients with pleural effusion at diagnosis, 10 relapsed (local 7, meta 3): 4/5 pts treated without RT relapsed locally versus 4/20 pts treated with additional RT. Positive pleural cytology doesn't seem to affect the incidence of reccurence.

At last follow-up, only 3/22 pts had impairement of quality of life (pulmonary restrictive disease).

Conclusion: Non metastatic EwFT of the ribs are curable by multimodality therpay and don't look like a bad prognostic location. Surgical resection is an important procedure before but more often after induction CT. RT can be avoided in some selected cases but should be given in all pts with pleural effusion, even with negative pleural cytology. In the SFOP experience, the sequelae of a multimodal therapy were moderate.

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O-200

EWING'S SARCOMA LIMB PRIMARIES IN PATIENTS AGED UNDER 5 YEARS: AN EICESS REVIEW OF 40 CASES.

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Ewing's sarcoma is uncommon in children under 5 years of age. Out of a combined data set of 598 patients with limb primaries registered with GPOH/CESS or UKCCSG/MRC trials offices 40 (7%) were under 5 years old. The aim of this review is to describe treatment and outcome with specific attention to local therapy issues related to treating young children with limb primaries. Out of these 40 cases 20 were femoral, 8 humerus, 8 tibia, 3 fibula, and 1 radius, 22 were male, 18 female, 4 had metastases at diagnosis. The patients were treated between 1980 and 1995. There are 29 patients surviving (1 still on treatment) with a median follow-up of 56 months (range 4 - 160 months). Overall prognosis was not different to that for older patients with limb primaries (5 year RFS 64% vs. 58%, p=0.9). Decisions relating to local therapy were made on an individual basis depending on site, age, and other factors. In the patients with lower limb primaries 9 had amputations, 7 had radiotherapy, 7 had resections, 4 had a combination of surgery and radiotherapy, 1 had no local therapy (PD), and there were 3 for whom local therapy data was not available. For those with upper limb primaries 4 had resections, 4 had radiotherapy alone, 1 had an amputation, and 1 was unspecified. The choice of which mode of local therapy to use in these patients is complex, given the potential side effects of radiotherapy or surgery to developing limbs; amputation has obvious costs. In the patients over 5 there has been an increased proportion of patients having resections (alone or in combination with radiotherapy); this accounted for 35% of limb primaries before 1986 and then 75% in the following period. There was no increase in resections in the patients under 5 years old. The use of endoprosthetic surgery is now common for older patients, in this series 3 children under 5 had an endoprosthesis, all with femoral primaries (ages 23, 29, and 48 months). Two of the 3 patients remain alive, one having a lengthening operation after 3 years; the other is only 16 months off treatment. To what extent such patients can benefit from advances in surgical techniques raises complex questions in quality of life issues which require further investigation. The long term consequences of each mode of local therapy has been reviewed and recommendations are made for future management of this difficult group of patients.

O-201

PHARMACOKINETICS, SAFETY AND EFFICACY OF DOLASETRON MESYLATE IN CHILDREN RECEIVING MODERATELY TO HIGHLY EMETOGENIC CHEMOTHERAPY

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In two identically designed, concurrently conducted pediatric studies, we evaluated the pharmacokinetics, safety and efficacy of dolasetron mesylate, a potent and selective 5HT3 receptor antagonist. An open label dose escalating design was used to assess the pharmacokinetic In two identically designed, concurrently conducted pediatric studies, we evaluated the pharmacokinetics, safety and efficacy of dolasetron mesylate, a potent and selective 5HT3 receptor antagonist. s, safety and efficacy of 0.6 mg/kg, 1.2 mg/kg, 1.8 mg/kg of dolasetron mesylate administration either intravenously (iv) or orally (po) 30 minutes prior to the start of chemotherapy. A dose of 2.4 mg/kg was also tested in the iv study. A total of 78 patients (pts) were studied (46 iv, 32 po); 56% were male (44/78). The median age of the studied group was 10.5 years (range: 3-17 years). Serial plasma samples were collected for 24 hours after dosing and the principle metabolite, MDL 74,156, was measured by HPLC. Emetic episodes, adverse events and use of rescue therapy were recorded in a 24 hour diary. Adverse events were mild to moderate in intensity and comparable to adult studies and could partially be explained by the patients' disease and/or cytotoxic therapy. The most frequent adverse events were tiredness and headache (both 15% of pts). As in adults, transient, clinically unimportant increases in mean ECG intervals including PR, QRS and QTc occurred. Non-compartmental pharmacokinetic parameters were estimated for MDL 74,156 in plasma and mean values from children were compared to data collected from a separate study of 18 healthy male volunteer subjects. MDL 74,156 plasma maxima were achieved within 1 h of dosing (0.5 h iv, 1 h po) and the mean plasma half life was found to be between 5 and 6 h, with apparent clearance values of 35 ml/min/kg and 16 ml/min/kg for the po and iv doses respectively. The 1.8 mg/kg (iv or po) dose yielded the highest complete response result. Conclusions: In children dolasetron mesylate was well tolerated over the 0.6 to 2.4 mg/kg dose range studied. Compared to adults the half-life of MDL 74,156 in children was 25 to 33% shorter. Finally, the apparent clearance was increased by 1.5-3 fold over values observed in healthy volunteers. While not a definitive study, 1.8 mg/kg, iv or po, appears to be the most effective dose.

O-202

CLINICAL USE OF TRANSDERMAL FENTANYL IN PAEDIATRIC AND ADOLESCENT PATIENTS WITH CANCER PAIN

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Transdermal fentanyl is a non-invasive approach to the treatment of opioid dependent cancer pain. The transdermal route has distinct advantages when oral administration of medication is difficult, due to local disease, gastro-intestinal disturbances or when side effects from oral drugs lead to poor compliance. Clinical trials in adults have demonstrated efficacy, an improvement in quality of life measures and a reduction in opioid side effects. These reported advantages, and the simple non-invasive method of delivery, encouraged our Paediatric Oncology Unit to pilot transdermal fentanyl in palliative patients with opioid sensitive pain. Since December 1994, 15 patients with relapsed malignancies or cancer related pain, have been treated with transdermal fentanyl. Patients with treatment related pain were not considered for therapy with fentanyl because of the potentially short length of opioid requirement. The age range of patients was 2 yrs 11 mths to 21 yrs 6 mths. All patients were previously receiving oral morphine in doses from 20mg/day to 360mg/day, and as a consequence of side effects related to oral opioids, or poor compliance with medication, were transferred to fentanyl. Treatment was commenced in doses according to standard oral morphine dosage conversion, and increased according to individual patient needs. Treatment was well tolerated and pain relief was good in all patients. Minimal side effects were experienced. Opioid related constipation, nausea and drowsiness was reduced with fentanyl, compared to that with prior oral opioid therapy. Compliance and patient satisfaction was high. We conclude that transdermal fentanyl offers an innovative approach to treatment of opioid sensitive cancer pain in paediatric and adolescent patients.

O-203

HOME-TREATMENT OF PEDIATRIC PATIENTS WITH CANCER : ECONOMICAL BENEFITS OF ONCE-DAILY ANTIBIOTIC TREATMENT.

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Health economics are becoming an important aspect of all medical care. Actually, febrile cancer patients with neutropenia are frequently hospitalized for intravenous antibiotic treatment. The development of antibiotics for which a once-daily administration is effective has broadened the 'home treatment' possibilities. At the University Hospital of Ghent a project named "Koester", was started to offer high qualitative curative and palliative care to pediatric cancer patients at home. Pediatric oncology nurses, working under the supervision of the pediatric oncologists, organize and coordinate home care in collaboration with the district nurses and with the general practitioner. Retrospectively we evaluated the economical benefits of once-daily intravenous antibiotic treat-ment at home, offered to pediatric oncology patients with infectious complications, which generally necessitate hospitalization. For various reasons it seemed more opportune for these patients, all with central venous system access, to offer them antibiotic treatment at home.

During the period 5/92-9/95, 26 patients were treated at home with once-daily intravenous doses of ceftriaxone and/or teicoplanin, depending on the proven or suspected microbial agents, during 375 days (mean 10,4 days/treatment period). The economical benefits for the Belgian health insurances were estimated at approximately 15O OOO US $, by saving the costs of the occupation of a hospital bed (400 US $/day x 375 days). On the contrary, the costs of home treatment were covered by the parents and by the Koester project, since no adequate structural financements of home care exist for the moment. The psychological benefits of 'staying at home' instead of 'staying in the hospital' for children with cancer and for their parents are evident. The Koester project now largely depends on private gifts. A prospective study of the feasability of the management of all febrile neutropenic patients at home has been initiated.

Conclusion : The management of the febrile neutropenic patient at home saves a lot of money for the health insurances and results in more satisfaction for the patient, the relatives and the health care professionals. It is our hope that health care authorities will take their responsibility to support a frame work of home care.

O-204

ONCE DAILY ANTIBIOTIC THERAPY OF FEBRILE EPISODES IN NEUTROPENIC CHILDREN

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The aim of this open prospective study was to investigate the possibility of treatment of febrile episodes in children and adolescents with chemotherapy-induced neutropenia or aplastic anaemia preferably in an outpatient setting. From Jan '93 until Nov '95, a total of 139 patients with 173 febrile episodes were included. Patients were 0.5 to 22 years of age with diagnoses of a solid tumour (47%), haematological malignancies (45%), and malignant lymphoma (8%). Inclusion criteria were granulocyte count below $1x10^9/l$, fever >38.5, and CRP >1mg/dl. Patients were eligible for outpatient therapy if they were clinically stable and from reliably compliant families. All patients received 80 mg/kg BW ceftriaxone (max 4 g) once daily as 30 minute infusion. For persisting fever or increased CRP elevation teicoplanin was added.

Single agent once daily ceftriaxone was sufficient in 109 (74.7%) of infectious episodes. Further 29 patients responded to the combination of ceftriaxone and teicoplanin to a total response rate of 79.8%. 35 patients were switched to an alternate antibiotic regimen. All febrile events finally resolved, there were no deaths. Hospitalisation was avoided completely in 28% of patients and partly in 37%. 35% of patients were hospitalised for the complete course of antibiotic therapy.

The use of ceftriaxone with or without teicoplanin seems appropriate for antibiotic coverage of febrile episodes in the majority of neutropenic patients and can be administered safely as outpatient treatment in clinically stable and compliant patients.

O-205

ALTERNATIVE TREATMENT FOR FEVER AND NEUTROPENIA IN CHILDREN WITH CANCER: ORAL CIPROFLOXACIN VS IV CEFTRIAXONE DELIVERED IN AN OUT PTS BASIS

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From Nov. 93 to Dec. 95, 138 consecutive episodes of fever (F+) and neutropenia (G+) were registered among 70 pediatric pts with solid tumors and stage I/II lymphomas. Children over 3 years of age were randomized to receive either oral ciprofloxacin (CP) 25 mg/kg/d or IV Ceftriaxone (CT) 100 mg/kg single dose daily as empirical antibiotic therapy when they presented F+ and G+ (<500/mm3). Of 116 evaluable episodes, 59 received CP and 57 CT. The median age was 10,3 y (CP) and 9,4 y (CT), range 3 to 20 y. The distribution of types of infection were: fever of unknown origin (FUO): 41% (CP) and 32% (CT); clinically documented (CDI): 56% (CP) and 63% (CT); and microbiologically documented (MDI): 3% (CP) and 5% (CT). The main sites of CDI for both group were sinus, mucosa, throat, ear, skin and gastrointestinal tract. Microbiological agents were identified in cultures collected from throat (*S. aureus, K. pneumoniae*, group B *Streptococci, S. viridans*) urina (*P. mirabilis*) and blood (*Aeromonas hydrophila*). Patients were treated in ambulatorial basis and hospitalization was necessary in 7% of those treated with CP and 4% of those treated with CT due to clinical worsening or necessity of adding other antibiotic or antifungical agent. The medium duration of G+ was 5,0 days (CP) and 5,9 days (CT). The duration of F+ ranged from 1 to 9 days for both groups, mean: 3,6 days (CD) and 3,2 days (CT). All patients stayed alive, 83% of those that used CP had sucess without modification vs 75% treated with CT. No side effects were noted in CT group while 8% episodes treated with CP were related with gastrointestinal symptons. None of them had bone or articular complains, neither radiological changes. The results of this study demonstrated the efficacy and safety of oral use of quinolon compound CP in selected pediatric cancer population with fever and neutropenia offering a new option of treatment delivered in an out patient basis.

O-206

AMPHOTERICIN B LIPID COMPLEX (ABLC) TREATMENT IN CHILDREN WITH INVASIVE FUNGAL INFECTIONS.

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ABLC (Abelcet™) is a lipid complex of amphotericin B which has been shown in adults to be at least as efficacious as amphotericin B (AmB) and substantially less toxic. We have retrospectively reviewed, for safety and efficacy analysis, 26 treatments given to 25 children in 6 centers in France. All had a proven invasive fungal infection : 16 aspergillosis, 7 invasive candidiasis, one trichosporonosis, one fusariosis and one non-identified filamentous fungi infection. Reasons for ABLC therapy were failure of previous amB therapy in 18 cases and contra-indication to AmB for renal impairment in 8 patients. Median age was 10 years (range : 6 months - 18 years). Underlying diseases were hematological malignancies (8 AML, 1 ALL), bone marrow transplantations (allogenic : 6 ; autologous : 4), solid organ transplantations (2), solid tumors (2) and aplastic anemias (2). All but 4 patients were neutropenic.

Mean daily dose of ABLC was 4.4 mg/kg (range : 1.7 - 9.9) with a median treatment duration of 47 days (range : 6 - 143). The median cumulative dose given was 4950 mg (range : 700 - 15,640 mg). Renal tolerance was excellent with a mean decrease in serum creatinine levels of 7 µmol/l. Eleven acute side effects (chills : 3 ; fever : 5 ; nausea : 1 ; vomiting : 1 ; thoracic pain : 1) have been recorded. All but two were graded as mild or moderate. No treatment had to be stopped for an adverse event. Clinical response defined as a cure or an improvement was observed in 23 patients (88 %). There was no difference according to the type of fungal infection (88 % in aspergillosis, 86 % in candidiasis and 100 % in other infections).

The excellent general and renal tolerance of ABLC and the satisfactory response rate in patients refractory or intolerant to conventional Am B are very promising.

O-207

A RANDOMISED TRIAL OF DOSE INTENSIFICATION IN OSTEOSARCOMA. AN EUROPEAN OSTEOSARCOMA INTERGROUP STUDY

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Previous EOI studies have shown that the two drug regimen cisplatin/doxorubicin (CDDP/DOX) is equivalent in both survival and relapse free survival to a more prolonged and expensive regimen containing methotrexate. Further analysis of the previous studies has shown improved outcome for those patients who received a greater dose intensity of CDDP/DOX. The 80931 study was therefore designed to test the hypothesis that increasing dose intensity will improve survival in osteosarcoma. Patients were randomised to receive CDDP/DOX at either 2 or 3 weekly intervals. Those who receive the drugs at a 2 week interval also receive GCSF. Definitive surgery is undertaken after 6 weeks in both arms i.e. after 2 courses of CDDP/DOX in the standard arm and 3 courses in the accelerated arm. The study was activated in June 1993 and by the end of February 1996 138 patients had been entered. Toxicity has been carefully monitored and there is no excess toxicity in either arm. Renal and cardiac toxicity have been especially closely monitored.

A total of 400 patients are needed to be entered into the study in order to have a 95% chance of demonstrating a 15% difference in survival.

New centres are welcome to enter patients into the study. Full details are available from:

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TREATMENT OF OSTEOSARCOMA WITH HIGH-DOSE METHOTREXATE-CONTAINING NEOADJUVANT CHEMOTHERAPY. SCANDINAVIAN SARCOMA GROUP DATA

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The Scandinavian Sarcoma Group (SSG) has conducted two consequtive trials for children and young adults (median age 16 years, range 2-39) with extremity-localized, non-metastatic disease. From 1982 to 1990, 102 patients (pts) were treated according to the T10 protocol (trial SSG 2), with pre-operative chemotherapy consisting of 4 courses of high-dose methotrexate (HDMTX) 8 g/m^2 at weekly intervals. In the subsequent SSG 8 protocol, 97 pts have been treated with the addition of 2 cisplatin/doxorubicin (CDP/D) courses pre-operatively. From 1993, the HDMTX dose was increased to 12 g/m^2. Median follow-up for surviving pts in SSG 2 is currently 106 months (range 59-163), and for SSG 8 31 months (range 3-66). 5-year metastasis-free survival (MFS) for SSG 2 is 56% and for SSG 8 63% ($p=0.50$), and overall survival (OS) is 66% and 73% ($p=0.09$), respectively. Poor responders crossed over to novel postoperative "salvage" chemotherapy (CDP/D in SSG 2 and etoposide/ifosfamide in SSG 8). Despite this, good responders had significantly better MFS ($p=0.003$) and OS ($p=0.0001$) than poor responders in both protocols. The addition of CDP/D to the pre-operative treatment in SSG 8 led to an increase in the fraction of good histological responders from 18% to 58% ($p<0.0001$). Significanly higher serum MTX levels in SSG 8 pts may also have contributed to this, as there was a significant positive correlation between serum MTX levels and histological response in both studies. This correlation was stronger when considering serum MTX levels after 24 and 48 hours, than at the end of the 4-hour MTX infusion, indicating that the rate of MTX elimination is of importance for the antitumour effect. Serum MTX levels at 24 and 48 hours were inversely correlated to hydration volume. There were no consistent direct correlations between serum MTX and survival, and no detectable variations in serum MTX with age.

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OSTEOGENIC SARCOMA (OS): RANDOMIZED TRIAL OF INTENSIVE PRE-OPERATIVE (PRE-OP) CHEMO VS CHEMO GUIDED BY HISTOLOGIC RESPONSE (HR) TO PRE-OP CHEMO.

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Patients (pts) with OS with a poor HR to pre-op chemo with bleomycin, cyclophosphamide, and actinomycin-D (BCD) and high-dose methotrexate (HDMTX) have a poorer prognosis for both event-free survival (EFS) and survival. This may be due to delayed introduction of alternative drugs with activity against OS, including adriamycin (A) and cisplatin (CDDP). We have conducted a prospective randomized trial. Regimen I calls for 10 weeks of HDMTX, BCD, A/CDDP (A 75 mg/m^2/72hr by continuous infusion, CDDP 120 mg/m^2) pre-op with the same agents continued after definitive surgery. Regimen II calls for 6 weeks of HDMTX + BCD pre-op; after definitive surgery pts with a better HR (Huvos Grade III or IV) received A + HDMTX; pts with a poorer HR (Huvos Grade I or II) received A/CDDP + HDMTX + BCD, with early introduction of A/CDDP. 73 evaluable pts are enrolled on the trial. One pt is inevaluable for HR; for 72 patients, Huvos response grade (# of pts):

	I	II	III	IV
Reg I	4	16	4	12
Reg II	9	13	9	5

Median follow-up is > 6yrs. For 12 pts with clinically detectable metastasis 5-yr EFS is 20%. For 61 pts without metastasis, 5-yr EFS is 75%. EFS correlates with HR. For HR I, EFS is 50%; II, 74%; III, 83%; IV, 88%. Actuarial EFS at 5 yrs is 73% for Reg I and 78% for Reg II. Reg I achieves more pts with Grade IV HR than Reg II but does not improve EFS. EFS for non-metastatic pts is identical to our previous T10 experience. Additional active agents or further increase in pre-op chemo intensity are needed to improve outcome.

O-210

NO EVIDENCE FOR IMPROVED EVENT-FREE SURVIVAL WITH PRESURGICAL CHEMOTHERAPY (PRE) FOR NON-METASTATIC EXTREMITY OSTEOSARCOMA. PRELIMINARY RESULTS OF RANDOMIZED PEDIATRIC ONCOLOGY GROUP TRIAL 8651, AN UPDATE.

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Improvement in event-free survival [EFS] of 20% has previously been attributed to the use of PRE in sequential trials in osteosaroma. In order to determine if PRE improved EFS, from Novermber ,1986 to December, 1993 we randomized 106 patients to immediate surgery [POST] or to presurgical treatment with two cycles [10 wks] of methotrexate, doxorubicin and cisplatinum. Except for timing, chemotherapy was identical for both groups with a planned duration of 44 weeks and included methotrexate, doxorubicin, cisplatinum, cyclophosphamide, bleomycin, and dactinomycin. Of the 106 patients 45 received PRE and 56 POST. Five patients proved ineligible and 1 had no evaluable data. Of these 100 evaluable patients 53 are male. The mean age is 13.6 [range 0.5 to 26.3yrs]. EFS and survival were analyzed to assess the impact of timing of chemotherapy [PRE versus POST]. Sixty - nine of 100 evaluable patients remain free from relapse or other failures [second tumors or death]. ALL EFS failures in both arms occurred by 4 years after diagnosis. With a minimum of two years of follow-up, the overall two year EFS is 72.5% [SE 4.8]; 71.6 [SE 7.2] for those randomized to PRE compared to 73.5% [SE 6.5] for POST [p = 0.60]. Five year EFS is 63.2% [SE 13.6] for PRE [30 of 45 patients remain failure free] compared to 65.5% [SE 11.6] for POST [39 Of 55 patients remain failure free]. The overall five year survival is 77.3% [SE 7.4] for all patients; 79.7% [SE 10.8] for PRE compared to 75.3% [SE 10.0] for post [p = 0.41]. We conclude that chemotherapy as given on POG 8651 was effective whether or not it is given initially or following surgery. In osteosarcoma there is insufficient evidence of an EFS or overall survival advantage for initial chemotherapy when compared to initial surgery.This comparison is estimated to have over an 80% power to detect a 20% difference.

O-211

METASTATIC OSTEOGENIC SARCOMA (OS) AT DIAGNOSIS. STUDY OF 73 CASES FROM THE FRENCH SOCIETY OF PEDIATRIC ONCOLOGY (SFOP) BETWEEN 1980 AND 1990.

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In order to assess the prognostic value of a good histological response (GR) in metastatic OS at diagnosis, 73 patients (pts) were retrospectively studied.
Patients : 36 boys, 37 girls, 3 to 19 years (med 13) of age, one case after bilateral retinoblastoma. The site of the primitive tumor (PT) was distal femur (40), proximal humerus (12), proximal tibia (11), other (10).
66 pts had pulmonary metastases (8 only one metastasis, 41 multiples pulmonary sites, 14 associed with bone, 3 combined sites). 5 pts had only bone metastasis. 2 pts had combined sites (liver, regional node).
Treatment (tt) : All pts received chemotherapy (CT) with (48) or without (25) Cisplatinum (CDDP); pts without CDDP received methotrexate and adriamycin as the main drug. Median duration of the first line CT was 7 months ; median number of CT courses was 11.
Local tt was possible in 60 pts : radiotherapy only in 18 pts (total median dose = 47 Gy), surgery in 52 pts (9 at diagnosis) ; surgical approach was mutilating in 32 pts, conservative in 20 pts, with a complete resection in 50/52.
The histologic response is evaluable in 41 pts : 21 GR (12 pts with CDDP, 9 without CDDP), 20 poor response (PR) (10 with CDDP and 10 without).
In 30 pts, lung metastases were resected (completely in 15 cases with 9 GR and 6 PR). 36 pts did not have thoracotomy because complete remission (CR) at chest-XRay (10) or progressive disease (21) or stable disease (5).
Results : 23/73 pts went CR for PT and metastases ; 15/23 pts relapsed : local (1), local with metastases (2) and metastases only (12). 43 pts died 2 months (mo) to 63 mo after diagnosis (med 15 mo), 17 are lost for follow-up with tumor after tt 3 mo to 31 mo (med 12 mo).
13 pts are alive in CR (lung 12, regional node 1) : CR1 (11), CR2 (2) after local relapse (1) or lung metastases (1) with a median follow-up of 7 y ; none of the alive pts had bone metastasis, all had surgery for the PT (GR 11, PR 1, non evaluable 1) and 6/13 had surgery for lung metastasis.Overall survival for all pts is 15% , for GR 50% and PR 5%.
Conclusions : 1 - this study confirms the poor prognosis value of bone metastasis at diagnosis (no patient alive). 2 - the survival depends on the achievement of CR and histological GR (12 alive pts /21 GR, only 1 alive /20 PR). 3 - 4/8 pts with only 1 pulmonary metastasis are alive ; the 4 dead had no surgery for their PT. 4 - surgery is necessary : all alive pts had surgery for the PT.

O-212

TREATMENT STRATEGIES AND OUTCOME IN METASTATIC (RELAPSED) OSTEOGENIC SARCOMA. THE SCANDINAVIAN SARCOMA GROUP (SSG) EXPERIENCE

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Since 1982, 199 patients (pts) of median age 16 years (range 2-39) with extremity-localized, high-grade osteosarcoma have been treated by two consequtive SSG protocols, both incorporating high-dose methotrexate, doxorubicin and cisplatin. The pts were recruited from 13 regional institutions in 3 Scandinavian countries (Country 1-3). For all pts, 5-year metastasis-free (MFS) and overall (OS) survival rates were 58% and 70%, respectively, with no significant differences between countries (OS for Country 1 69%, for Country 2 74%, and for Country 3 65%). 75 pts have developed metastases (30 from Country 1, 24 from Country 2, and 21 from Country 3), of these 90% had lung lesions (38% bilateral, and 15% in combination with other sites). Median time to the first metastatic event was 18 months (range 1-117 months), and the median number of metastases was 3 (range 1->10). There were no significant differences between countries as regards relapse-free interval, or number or site of metastases. 2-year OS from the first metastatic event was 30%, with significantly better survival in Country 2 (46%) than in Country 3 (12%) ($p=0.01$), and with intermediate results for Country 1. Improved survival was obtained in pts who had less than 3 metastases ($p=0.002$), who underwent complete metastasectomy ($p=0.0000$), and who received aggressive second line chemotherapy ($p=0.02$). The difference noted between countries in survival after metastases could in part be attributed to differences in treatment approaches and aggressiveness at the first metastatic event, as well as to differences in the performance of re-thoracotomies on subsequent metastatic episodes. It is conluded that the therapy delivered for metastatic relapse is of importance for overall outcome, and that collaborative osteosarcoma protocols should include protocolled salvage therapy for the treatment of relapse.

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REPORT FROM THE EUROPEAN INTERGROUP COOPERATIVE EWING'S SARCOMA STUDY (EICESS 92).

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Based on similar treatment approaches in previous trials, the German GPOH and the UK CCSG/MRC since 1992 have started a collaborative Ewing's sarcoma trial including two different randomisations for standard risk (<100 ml tumour volume) and high risk (≥100 ml tumour volume) patients (pts). The SR randomisation was for cyclophosphamide instead of ifosfamide following induction in combination with vincristine, actinomycin D, and adriamycin (VAIA vs. VAIA/VACA), the HR randomisation compared additional etoposide (EVAIA) to standard chemotherapy (VAIA).
The study is ongoing, results according to the randomisations are blinded. From July '92 until Feb '96, 527 pts were enrolled, 323 were randomised protocol pts, 72 SR and 251 HR, 204 follow-up pts. According to histology, 188 were rated as classical Ewing tumour, 36 atypical Ewing tumour, 79 PNET, 25 other small round cell tumours. 233/323 pts were registered as non-metastatic, 84/323 as metastatic at diagnosis: lungs 33 pts, bone/bone marrow 27 pts, lung and other sites 11 pts, other sites 13 pts. The four major primary sites were pelvis 27%, chest wall 18%, femur 20%, and tibia and fibula 13 %. To date, 220/323 protocol pts have completed local treatment: 47 (21%) radiotherapy, 173 (79%) surgery, of those without radiation 49 (22%), followed by radiation 30 pts (14%), and following radiation 92 pts (42%). The histologic response was available in 126 pts, of those in 56 pts after presurgical chemotherapy and 70 pts after presurgical chemoradiotherapy. Following chemotherapy the good vs. poor response ratio was 70 vs. 30% compared to 81 vs. 19% following chemoradiotherapy. To date, 55/323 pts have relapsed, 46 systemic failures, 5 local failures, 4 combined local and systemic failures. 36 failures occurred in pts with primary disease at diagnosis, 19 failures in pts with metastatic disease at diagnosis. Definite results regarding study aims and objectives are not available at this stage.
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TREATMENT OF NON METASTATIC SOFT TISSUE PNET/EXTRA OSSEOUS EWINGS SARCOMA (EOES) : THE EXPERIENCE OF THE SIOP MMT 89 STUDY.

MCG Stevens, A Rey, M-T Praquin, O Oberlin for the SIOP MMT Committee.

The use of anthracycline drugs, with alkylating agents, is generally accepted as optimal chemotherapy for Ewings Sarcoma (ES) of bone. There is no consensus that the same approach is necessary for soft tissue PNET/EOES. These rare tumours may be clinically indistinguishable from rhabdomyosarcoma (RMS) despite evidence for their biological similarity to ES of bone, although they are unusual at favourable sites (as defined for RMS).

In the SIOP MMT 89 study, 276/1013 (27%) patients (pts) entered on protocol had non RMS soft tissue sarcoma. PNET/EOES accounted for the largest group (25%). All cases of non metastatic (Stages I - III) PNET/EOES, confirmed by pathology review and followed for >2 years were included in the analysis.
Stage I + II = 30 ; Stage III (node positive) = 4.
Site = Limbs (9) ; PM (7) ; Thorax (6) ; Abdo/pelvis non GU (4) ; NPM (3) ; Other (5). Altogether 75% were at unfavourable sites.
All pts. received initial chemotherapy (CT) with IVA (Ifosfamide 9g/m^2/course, Vincristine, Actinomycin D). Local therapy (RT ± surgery) was given according to response and site. Second line CT was Vincaepi (Vincristine, Carboplatin, Etoposide).

Overall survival and EFS were 63% and 46% at 5 years. 5 pts. (15%) failed to achieve CR ; all died. 6 (18%) had local relapse (LR) ; 4 were alive in 2nd CR at median 42 months. 5 (15%) had metastatic relapse (MR) ; 2 were alive in 2nd CR at median 19 months. 18 pts. (53%) were alive in 1st CR at median 33 months ; only 8 had received RT.

These results suggest that pts. with non metastatic PNET/EOES can be treated effectively within a soft tissue sarcoma strategy, without anthracycline as part of primary treatment. The possibility of sustained 2nd CR in 6/11 relapsed pts. (4 LR, 2 MR) is consistent with the observation from MMT studies that effective salvage is possible, particularly without previous RT. This experience challenges the view that the histological and molecular genetic definition of this diagnosis requires the same treatment as ES of bone.

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MALIGNANT PERIPHERAL NEUROECTODERMAL TUMORS (MPNT) AND EXTRAOSSEOUS EWING SARCOMA (EES) IN CHILDHOOD AND ADOLESCENCE: RESULTS OF THE GERMAN COOPERATIVE SOFT TISSUE SARCOMA STUDIES CWS-81 + 86.

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EES and MPNT are histogenetically and cytogenetically related. There are however no definitive histological diagnostic criteria to differentiate between these two tumor types. Because the incidence of these tumors is low, there is little known about the optimal therapy strategy. 64 pts, >1 and <21 years of age with EES and MPNT were entered between 1981 and 1990 in the CWS-81 and 86 studies. The diagnosis of MPNT was made if at least one neural marker and/or Homer-Wright rosettes were present. According to this classification system there were 26 EES and 38 MPNT cases. The chemotherapy (CT) used in the CWS-81 consisted of CYC, ADR, VCR and AMD (VACA). In the CWS-86 study, CYC was replaced by IFO (VAIA). Pts with primary resectable tumors received adjuvant CT and RTX. Pts with a non resectable tumor mass at presentation with or without metastasis received neoadjuvant CT. For the definitive local tumor control RTX and/or second look surgery were performed after 3 (7-10 weeks, CWS-81) or 6 courses (16-20 weeks, CWS-86) of CT. In the CWS-81 study, RTX was conventionally fractionated while in the CWS-86 study, it was accelerated hyperfractionated (2x1,6 Gy/day). In 20 pts the tumor was primarily resected (St. I+II). 10/20 were irradiated with 32-60 Gy. 15/20 (75%) pts with St. I and II are in 1.CCR median 81 months (18-155), 9/10 who were irradiated and 6/10 who did not receive irradiation. 44 pts had primary non resectable tumors, 16 with distant metastasis. 37 pts were evaluable for response to preoperative chemotherapy. In 1/9 (11%) pts treated with VACA and in 19/28 (67%) treated with VAIA greater than 2/3 reduction in tumor volume after 3 courses of CT was seen. 24/28 pts in St. III were irradiated with 32-60 Gy. The median radiotherapy dose for patients with primary localised tumors was 50 Gy in the CWS-81 study and 32 Gy in the CWS-86 study. 12 out of 28 patients (43%) in St. III and 2 out of 16 (13%) pts in St. IV are alive in 1. CR 24 to 143 months. There were 15 relapses in pts with St. I-III: 7 local, 4 distant and 4 combined. The EFS rate at 3 years for all pts with St. I-III was 55 ± 14% and 65 ± 8% in the CWS-81 and -86 studies respectively. There was no significant difference in prognosis between patients with EES and MPNT: 15/26 (57%) with localised EES and 15/38 (39%) with localised MPNT are disease free for 18-155 and 26-108 months respectively. In summary: the improved tumor response to initial CT with VAIA in comparison to VACA did not alter significantly the final therapy results results. However, the RTX-dose was reduced in the CWS-86 in comparison to CWS-86.(supported by BMFG and Deutsche Krebshilfe)

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NON METASTATIC RHABDOMYOSARCOMA (RMS): OUTLINES OF THE SIOP MMT 95 STUDY.

O.Oberlin, A.Rey, MT Praquin, MCG.Stevens for the SIOP MMT Committee.

The concept for this study has been built on the experience of SIOP MMT84 and MMT89 studies which both explored the use of chemotherapy (CT) to restrict, where possible, the systematic use of definitive local therapy (surgery or radiotherapy) in an attempt to reduce the risk of important late functional and cosmetic sequelae.

The principle aims of the study, defined according to risk groups are:

- for the low-risk patients (pts) (localised and completely resected primary at all sites), to maintain the excellent survival and to improve disease-free survival by a more precise pre treatment staging and more careful assessement of completeness of initial surgical resection. These patients only receive Actinomycin D + Vincristine (in MMT89, 3 yr EFS = 76 %, Overall survival = 91 %).
- for high-risk pts (non metastatic pts with incompletely resected tumours at all sites except vagina or paratestis), to compare the efficacy of first line CT using IVA alone with a more 6 drug combination, but without changing the approach to local therapy (in MMT89, using CT with IVA, 3 year EFS = 68 % and OS = 86 %). Local treatment depends on the site of the tumor and response to initial CT: Only pts with parameningeal tumor aged > 3 year and pts who fail to achieve complete remission with CT at other sites will be given local therapy.
- for stages III (postive nodes), to maintain the improved outcome for these patients treated in MMT89 with a 6 drug combination but to reduce the duration of chemotherapy from 12 to 9 course (in MMT89 3 yr EFS was 55 %, OS was 64 %).

The MMT95 study will be conducted in relationship with the German and the Italian cooperative groups for MMT. All these studies will address the question of chemotherapy in a randomized trial for high risk patient groups. They will compare a common arm (the 6 drug arm) to their reference arm (IVA for SIOP, VAIA for the German and Italian groups).

The SIOP MMT95 study has been opened on 1st July 1995.

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FIRST RESULTS OF A MULTICENTER TREATMENT STUDY FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH A MODIFIED BFM-90 PROTOCOL IN THE UKRAINE

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The UGCL was established in Jan. 93, when 9 Ukrainian Pediatric Centres (Kiev City Hospital, Kiev Regional Hospital, Lwow, Donezk, Dnjepopetrovsk, Ternopol, Simferopol, Charkow) combined their efforts for treatment of children with leukemia with support by German specialists (G. Schellong, Münster; A. Reiter, Hannover). The laboratory for cytochemistry in the Inst. of Pathol., Oncol. and Radiobiol. of the Ukrainian Academy of Sciences is working as diagnostic reference laboratory (D.G.). The first protocol, dedicated to ALL-therapy, was based on the German ALL-BFM-90 protocol. The main modification consisted of the replacement of 1 g for 5 g/m^2 MTX in protocol M. High risk (HR) pats were treated according to the treatment schedule for medium risk (MR) pats. Between Nov. 93 and May 95, 182 children and adolescents (age 10 mths to 17 ys 11 mths) with previously untreated ALL were enrolled in the UGCL study. 8 pats died during induction, 2 were non-responders. CR rate was 94.5 %. As of Dec. 31, 1995, 12 pats had died in remission (predominantly due to infections and hemorrhagic complications) and 2 pats. suffered relapse. The probability for EFS at 25 months is 78 % (SD 4 %) and for survival 82 % (SD 3 %). The relatively high rate of fatal complications (11 %) reflects the limited possibilities in supportive care and the not yet optimal hospital structures in the successor states of the former Sovjet Union. Nevertheless, the preliminary results of the project reveal a dramatic improvement of the outcome of ALL in the Ukraine after introduction of the modified ALL-BFM-90 protocol on a nation-wide cooperative basis. Recently 2 further protocols (AML, ALL-Relapse) and guidelines for supportive care for oncohematologic pats have been worked out.

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THERAPEUTICAL RESULTS IN ALL

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Between Nov. 1987 and Dec. 1994, 230 patients with ALL were treated in Children's Oncohaematology Clinic in Sofia according to Protocol 01-81 of Dana Farber Cancer Institute (Boston - USA). Patients' median age was 5.83 years; girls to boys ratio was 1:1.47; Standard risk (SR) patients to High risk (HR+VHR) was 1:1.61. Before the 7-th day of Induction therapy there were 20 early deaths. At the end of the Induction phase in 96.66% of the patients CR was achieved. A follow up longer than 3 months (max. 84 months) was possible in 173 patients (14.78% of the total number were withdrawn from the study due to various reasons). During the period of observation 13.29% of the patients (23 pats.) had Haematological relapses; 6.36% (11 pats.) - Meningeal and 5.94% (6 boys) - Testicular relapses. The probability for DFS at 5 years, according to Kaplan-Meyer's method, was 68%±6% for the total group; 78%±8% for SR group and 60%±10% for HR+VHR group.

The therapeutical results are very encouraging compared with the results in the past. The Protocol was very well tolerated and comparatively easy has been executed. Remission failures, the reasons for early deaths, failures to follow-up and relapses are discussed.

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TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN ESTONIA IN NINETIES: FIVE YEARS EXPERIENCE OF INTENSIVE CHEMOTHERAPY PROTOCOLS.

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In seventies and eighties only less than 1/3 of children with ALL achieved 5 years survival in Estonia. At the beginning of nineties special pediatric oncology units were opened. The aim is to analyse the problems of using intensive chemotherapy protocols in one of the developing countries, to compare remission rate and improvement of survival. 48 children up to the age 15 years were diagnosed as ALL during the 5 years period from January 1, 1991 to December 31, 1995 in Estonia. In 1991 and 1992 all children were treated uniformly using the BFM-ALL intermedium risk protocol with high dose Methotrexate (HDMTX) 1 g/m^2 and cranial irradiation after the second induction. Since 1993 immune-phenotyping of blast cells became available in our laboratory, the infectious control of neutropenic patients improved and we started to use central venous catheters. Since 1993 the children have been treated by modified NOPHO-ALL intermedium (IMR) and high risk (HR) protocols according to the risk-group. Patients were divided into two risk groups. Criteria for HR-ALL were age less than 2 years and more than 10 years at the diagnosis, WBC count more than 50x10^9/l, evidence of mediastinal mass, CNS involvment, T-cell ALL and no remission at 29th day of treatment. Our main modification in using NOPHO-ALL protocols was reduced MTX-dose: 1g/m^2 and using cranial irradiation. 45% of our patients had HR-ALL. The first complete remission (CR) was achieved in 90%, 10% of patients died during the first induction therapy without remission. The main courses of death were infections and haemorrhage. 23% ALL patients have relapsed and 67% of children have the first CR. Relatively high rate of CNS relapses shows that HDMTX more than 1g/m^2 is needed. We hope to start using MTX 5g/m^2 in 1996 when MTX serum level measurement will be available in our laboratory.

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RANDOMIZED COMPARISON OF TWO PROTOCOLS FOR CHILDHOOD ALL IN RUSSIA: MB 91 AND MODIFIED ALL BFM 90

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At the XXVIth SIOP Meeting we reported encouraging results with a protocol designed for treatment of childhood ALL in Russia: Moscow-Berlin (MB) 91. Major aims were (1) to use presumably less myelotoxic therapy requiring less supportive care, blood (component) transfusions and hospitalization, (2) to avoid potentially hazardous high-dose therapy, (3) to avoid preventive CNS irradiation in the majority of patients (pts), and (4) to limit the use of anthracyclines. Since 1991, 107 pts have been treated at the Moscow Institute of Pediatric Hematology. The remission rate was 97%. Kaplan Meier estimate for EFS at 4.5 years (median follow up 18 months) is 0.87 ± 0.04.

In January 1993, a randomized trial was started to compare MB 91 with a slightly modified version of the German protocol ALL-BFM 90 (1g/m^2 instead of 5g/m^2 of systemic methotrexate). Until November 1995, 144 children (MB 91: n=73; BFM: n=71) were enrolled from 4 participating centers. There were no significant differences among both groups regarding sex, age, WBC, occurrence of T-immunology and CNS involvement. Remission rates were 96% with MB 91 and 100% with ALL-BFM 90. The estimates for EFS at 3 years are 0.86 ± 0.6 (MB 91) and 0.85 ± 0.6 (ALL-BFM 90). The median follow up of both groups is 11 months. Like in the total group, treatment results are also not different regarding pts at lower or higher risk for relapse. Major aims of MB 91 could be achieved.

Conclusion: Protocol MB 91 has been designed with respect to specific conditions in Russia. These are to avoid hospitalization, frequent blood transfusions and central venous catheters as potential sources of infections requiring extensive and expensive supportive care. Avoidance of high-dose therapy, CNS irradiation and limited use of anthracyclines reduce the risks of acute and late toxicity. These are aims for every modern ALL treatment, not only in Russia. This interim analysis shows that treatment results with MB 91 are comparable to an international standard (ALL-BFM 90).

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O-221

IMPACT OF EARLY INTENSIFICATION THERAPY ON CLINICAL OUTCOME IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA: COMPARISON OF CCSG 105 WITH AND WITHOUT INTENSIFICATION THERAPY.

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The impact of early intensification therapy on survival in Acute Lymphoblastic Leukemia was studied and compared to treatment outcome without intensification. Of the 148 patients between the ages of 0-14 years evaluated during the period 1987-94, 122 patients (83.43%) were inducted in this study. Sixty patients (49.18%) were treated with the multi drug regimen CCSG 105 without intensification therapy while 62 patients (50.82%) were treated with the CCSG 105 with intensification therapy following Interim Maintenance Phase.

In the group treated with CCSG 105 without intensification 91.6% patients went into complete remission after induction with the triple drug regimen using Vincristine, Prednisone and L-Asparaginase. We observed a high incidence of Bone Marrow (29%) and CNS (20%) relapse during maintenance and after completion of therapy. In this group 32.7% patients remained in complete remission having completed 3 years of treatment. The median duration of survival was 22 months with maximum observation time being 84 months.

In the group treated with CCSG 105 with intensification therapy following Interim Maintenance Phase, 98% patients were noted to be in complete remission following induction with a four drug regimen employing Vincristine, Prednisone, L-Asparaginase and Daunomycin. In this group 59.67% patients remained in complete remission with median survival of 40 months. The maximum observation time was 62 months.

We conclude that the survival outcome and remission rate improves significantly with early intensification therapy and use of four drugs for induction

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AN AUDIT OF CHILDREN WITH CANCER TREATED FOR FEVER AND NEUTROPENIA : COSTLIER THAN CYTOTOXIC CHEMOTHERAPY

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Children with cancer are often hospitalized for chemo- and/or radiotherapy-induced fever and neutropenia. An audit of fever and neutropenia was performed to assess the clinical efficacy and cost effectivity of the policies on antibiotics use. We reviewed records of 117 consecutive hospitalizations for fever and neutropenia involving 44 patients (pts) under 19 yrs of age and a period of 36 months. Thirty-one pts had leukemia or lymphoma and 13 had solid tumors. Infection was clinically documented in 61% of the episodes and microbiologically proven in 24%. Common sites were the respiratory and GI tract. Nineteen bacteriemias (16.2%) were documented. Commonest organisms were Staph. 46.3%, Pseudomonas sp.21.4%, E.coli 21.4%, and K.pneumonia 10.7%. Fever of unknown origin occured in 15.3% of the episodes. Second-line therapy for failure to control infection was vancomycine at 72 hrs.Amphotericin-B was introduced for persistent fever at the 96th hour. Thirty-three episodes were treated with piperacillin + amikacin , 32 with mezlocillin + amikacin, and 28 with imipenem + amikacin. The overall clinical efficacy rates with these antibiotic combinations were 27.3%, 36.7% and 53.9% of the episodes, respectively. Antifungal therapy with fluconazole /amphotericin-B was given in 33 episodes. Mortality rate was 4.3% per episodes. There were three deaths directly attributable to the infective episodes and two to toxicity. The median duration of fever was four days and the average length of hospitalization was ten days. An avarage, total cost of hospitalization including antibiotic and growth factor, was US$ 1527 per episode. The average costs of the antibiotic therapy for an episode were US$1388, 835, and 865 for the years 1993, 1994 and 1995 respectively. These results show that imipenem + amikacin (used predominantly during 1995) may be an effective therapy in selected patients. Introduction of early emprical antibiotic therapy has decreased the mortality rates attributable to infection.

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ADVOCACY FOR A COMFORT TREATMENT FOR CHILDREN OVER 1 YEAR OLD WITH STAGE IV NEUROBLASTOMA

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Metastatic neuroblastoma (IV NB) of the child over 1 year (yr) old has very poor prognosis despite intensive chemotherapy (CT). Bone Marrow Transplantation (BMT) and immunotherapy have slightly improved the survival of these children. All these treatments (trt) are costly, painful and hard for both children and parents. Morocco, as a developing country (DC), is unable to face such great cost for so few results. We would like to report our experience in stage IV NB which could interest doctors from other DCs. In our unit, over 12 years, we registered 100 cases of stage IV NB over 1 yr old. Ages ranged from 13 mths to 14 yrs (average 43 mths), sex ratio was 1,5. Most patients (pts) presented with anemia (60), bone pain (40) or Hutchingson syndrom (29). The site of the primary tumor was abdominal (69), mediastinal (3), pelvic (3), cervical (2), spinal (3) and unknown (20). Metastaic sites were mainly bone and bone marrow (82); other sites were nodes (15), skin (8), liver (7), lung (3) and brain (3). Only 80 pts were treated: 2 of them were sent to France where they recieved HD CT followed by BMT and IL2; 32 were treated according to CADO procole (Vincristine 1,5 mg / m2 d1 and d5; Cyclophosfamide (CPM) 300 mg / m2 d1 to d5; Doxorubicine 60 mg / m2 d5; 6 to 8 cycles with 3 weeks intervals), 46 recieved CPM alone per os 1ϕ mg / kg / d, 1 week on, 1 week off, as long as well tolerated and efficient. Results were as follows: The 2 first pts had neurologic and hepatic toxicities, a partiel remission and died after 10 and 12 mths. They spent 8 mths in France and during this period, their parents had to leave their job. The two other groups (CADO and CPM) had the same response concerning immediate efficiency but more toxicity with CADO. Besides, the longest survival was seen with CPM (36 mths). The mean duration of hospitalisation was 5 days for the pts recieving CPM and 50 days for those treated by CADO. The advantages of trt by CPM are numerous: it is well tolerated, not painful, not costly, it can be taken at home, it doesn't disturb the social life of the family. So, it seems clear that CPM, in default of curing the pts, provides them a good quality of life which is important to take into account. This paper is an advocacy for a good quality of life for ill children and for a pragmatic pediatric oncology for DCs which cannot reasonably use their few means for an almost hopeless disease.

O-224

RETINOBLASTOMA (RB) EXPERIENCE IN A DEVELOPING COUNTRY

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From 1991 to 1994 hundred and twenty patients with RB were admitted at Pediatric Department of A. C. Camargo Hospital, Sao Paulo, Brazil - Median age at diagnosis was 24 months.
63 female and 57 male. Extraocular disease in 29 patients and intraocular in 91. Bilateral tumors in 56 patients and unilateral in 64. Patients with group V Reese Ellsworth were randomized to receive or not chemotherapy with Cyclophosphamide and Vincristine after enucleation. 20 patients were treated only with surgery and 24 received chemotherapy. Overall survival at 24 months was 95% for the group that received only surgery and 92% for the group that received surgery plus chemotherapy (p=0.749).
Overall survival was 98% and 96% at 12 and 24 months respectively for patients with intraocular disease and 68% and 55% at 12 and 24 months for patients with extraocular disease.
New agents are needed to improve survival in patients with extraocular disease.

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CHILDHOOD THYROID CANCER AFTER CHERNOBYL ACCIDENT IN RUSSIA

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The number of children with thyroid cancer has been increasing since the Chernobyl accident. In Scientific Research Institute of Pediatric Department of Russia were operated 212 patients (pts), 27 of whom were from Chernobyl area. The ratio of boy/girl is 2.4:1.5 and the number of children at the different age group was as follows: 5 under 7, 6-under 10, 10-under 13 and 6-under 15 years old. It took from 3 to 24 months after the final diagnosis (average-3 months) to receive an operation. We found out in children following symptoms: asymmetry of neck and thyroid region-25, irregular contours-4, solidification-18, change of shape-3, dyspnea, cough, aphonia, pain-in 1 case, respectively. In 11 cases there were unilateral and in 4 cases-bilateral neck lymph nodes metastasis (mts), in 3 cases-distant pulmonary mts. In 11 pts mts were found simultaneously with the primary tumor, detected 6 patients had mts before primary diagnosis, in 1 pt mts were found during surgery. It was mentioned the number of well-differentiated types of cancer: papillary-22, follicular-4, meddullary-1 case. In 9 cases the removal of primary tumor was performed, in 11 cases the removal of both primary tumor and mts, in 5 cases surgery was not radical. Hemithyroidectomy was performed in 1 pt, hemithyroidectomy + isthmus resection-in 12 pts, total thyroidectomy-in 8 pts, neck dissection-in 18 pts. 9 pts after surgery were given radioiodine (I-131). All children have been alive for 3 months-8 years since operation.

The influence of radiation on the above thyroid cancer is still unknown, however, the aggressive and younger manifestations are characteristic in childhood thyroid cancer around the Chernobyl.

O-226

HIGH GRADE OSTEOSARCOMA (OS) OF EXTREMITIES. RESULTS WITH MODERN THERAPY IN A DEVELOPING COUNTRY.

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Osteosarcoma is the malignant solid tumor most frecuently diagnosed in adolescents in our hospital. The outcome in the past, with radical amputation and adyuvant chemotherapy was poor. With the aim to improve survival and facilitate limb sparing procedures, a protocol with preoperative chemotherapy (PC) was designed. Since October 1991, 39 patients (pts), 19 boys and 20 girls, with OS (femur 18, tibia 14, humerus 5, fibula 2) aged 6 to 19 years (m 13.5 y) were elegible for limb salvage surgery and received PC with 3 cycles of Doxorubicin$_2$ (DOX) 75mg/m^2/24h infusion and Ifosfamide (IFX) 2gr/m^2 days 2-6 every 3-4$_2$ weeks, followed by 4 cycles of$_2$ Cisplatin (CDP) 150mg/m^2/intrarterially (IA) or 120mg/m^2/IV every 2 weeks. Postoperatively 4 to 6 cycles more were given. Twenty two pts received CDP IA and 17 pts IV. Limb salvage was possible in 35 pts (89.7%), Six pts had an amputation after conservative surgery due to local relapse in 4, and surgical complications in 2 pts. Three pts refused surgery and are excluded for survival analysis. Thirty six surgical specimens were analyzed according to Ayala's method, and 24 pts (66.7%) were good responders. The median follow up is 23.7 months (range 6 to 51m). The overall survival is 78.9m and event-free survival is 74.5 at 40 m (Kaplan-Meir).
We demonstrated that limb salvage in OS is feasible, the survival improved dramatically when compared with our prior experience. There are still complications derived from surgery and chemotherapy that must be diminished in further studies.

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TOWARDS CURING CHILDREN WITH CANCER IN DEVELOPING COUNTRIES WITH LIMITED RESOURCES - THE MALAYSIAN PERSPECTIVE

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This paper outlines our efforts to increase cure rates of childhood cancer in Malaysia, a rapidly developing country with about 550 new cases of cancer per year among this 6.9 million (37% of the total population of 19 million) children below the age of 15. Although childhood cancer are now largely curable, many especially those in less developed nations are still not cured. New knowledge to improve prognosis of childhood cancer has greatly increased but patients will only benefit if there are health infrastructure services relating to adequate staffing, equipment and other facilities. These are often limited and expensive in less developed countries where cancer is usually not a health priority in budget allocation. However, much can be achieved through good organisation, planning and marshalling of available community resources. Our efforts include the following:

1. Organisation of all 9 paediatric oncologists in the country into a cohesive Malaysian CCSG for collaborative research studies. National protocols have been developed for Wilms Tumour, Malignant Germ Cell Tumour and Hepatoblastoma.
2. Computerization of our epidemiologic and research data as performance indicators using commonly available application softwares customised with help of voluntary computer programmers.
3. Establishment of day-care facilities and half-way houses, largely through the efforts of voluntary service organisations.
4. Setting up of leukaemia and childhood cancer funds from public donations to help poor patients pay for their treatment.
5. Training of medical and nursing staff through organisation of courses. Emphasis is given to the more cost-effective method of bringing foreign specialists to the country rather than sending trainees overseas.

Future efforts include:

1. Developing national protocols for all types of childhood cancer.
2. Joining international collaborative research studies.
3. Establishment of regional childhood cancer centres to decentralise services.
4. Training to increase the humanresource pool to treat childhood cancer.

Much can be achieved to improve the prognosis of childhood cancer in less developed countries even with the optimal utilisation of existing resources.

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MEDICAL AND SOCIAL CARE OF CHILDHOOD CANCER IN MOROCCO

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Pediatric oncology (PO) has seen dramatic improvements in biological and clinical research. In 1995, in developed countries, 3 out of 4 children suffering from cancer are cured with a good quality of life. In Morocco, until 1980, there were no medical units treating childhood cancer. A child diagnosed with cancer was condemned to die except when his tumor was very localized or his family was financially able to send him to a French PO center. The Pediatric Hemato-oncology Unit (UHOP) has been created with 6 beds in 1983, inside a general pediatric department of the Children Hospital of Rabat, by a few nurses and doctors. In 1986, this unit had 20 beds and 34 beds in 1995, 8 of which are reserved for outpatients. Staff number has increased over the years but remains insuffisant. So, the children are often hospitalized with their mother or grandmother. This unit is sustained by the Association of Parents and Friends of Children with Cancer. The l'Avenir Association (the future) was born in 1986 thanks to the efforts of parents and medical staff. It aims at improving the medical and social care to the children suffering from cancer in Morocco. This association has built a house for parents living far from Rabat; it is member of the International Confederation of childhood cancer Parent Organizations (ICCCPO). Actual data of the PO in Morocco are as follows: The number of annual new cases is estimated at 1200, equally distributed between the northern and southern parts of the kingdom. The UHOP of Rabat takes care of patients (pts) from all the northern part. In 1983, there were 59 new cases and 268 in 1995. Only 15 % live in Rabat and only 15 % have a medical insurance. Ages range from 1 month to 17 years (average 7y), the sex ratio is 2. Most pts have NHL (28 %) or leukemia (22 %). Brain Tumors are not treated in our unit. The initial status of the patients is sometimes very advanced, less often in 1995 than in 1983. We treat patients according to protocols drawn from the SIOP and SFOP ones. Overall Survival rates are about 60 % for pts treated in UHOP. An informal but efficient partnership exists between Rabat's unit and several French units. Problems remain the early deaths and lack of pts follow up. The solutions are to increase the number of staff members, to improve the Children Hospital's laboratory and to improve communication.

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MALIGNANT PAEDIATRIC TUMORS:the experience of the Baca Ortiz Children Hospital 1993-1996

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Paediatrics patients below 18 years old refered our Oncology serices with malignant tumors have been reviewed. The aim of study was to document presentation,management and outcome and to compare theses parameters with those reported in the same population before April 1993. The study population consisted in 52 patients studied between May 1993 and January 1996.Being sex ratio M/F1.73 age more frequent of 1 and 4 in 46.1% and 10 and 14 years old in 21.1%. Diagnosis in100% were confirmed by histology. This population showed an incidence as follow: CNS tumor 36.5%,Wilms tumor 21% Osteosarcoma 13.4%,Germinal cell tumor 9.6%,Neuroblastoma 7.6%, Hepatoblastoma 5.7%, Ewing tumor 1.9%, Rhabdomiosarcoma 1.9%, and other 1.9%.As - special characteristic of study ,Astrocitoma represented the more frequent tumor of CNS;28.5% of children afected of Retinoblastoma had a bilateral presentation and a congenital neuroblastoma was described. Actuarial survival at 3 years was of 80.7% wich compares very favourably with the outcome of children with the same pathology overseas.except inthe Retinoblastoma group that was less favourable because the stage III and IV at diagnosis , being actuarial survival of 57%.Percentage of distant metastasis have decreased, because early diagnosis, and oportune treatment with chemotherapy with SIOP protocols.In conclusion morbimortality have decreased,survival rate have been improved , exist more confidence of population resulting in a better Oncology practice .

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ESTABLISHMENT OF A PEDIATRIC ONCOLOGY PROGRAM IN GUATEMALA.

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Despite the significant advances in Pediatric Oncology throughout industrialized countries, improved survival rates have not been in documented in developing countries such as Guatemala. In Guatemala the incidence, types and outcomes of pediatric cancer are unknown. Accessibility to chemotherapy and followup care is difficult. Government support for the study, diagnosis and treatment of childhood cancer is minimal. However, the experience of local oncologists suggests a higher incidence of T-cell leukemia, retinoblastoma, Ewing's sarcoma and Hodgkin's disease at a young age than that of developed countries. Oncologists, radiation therapists and nurses of the University of California, San Francisco and Guatemala formed a partnership in 1995 to work toward the establishment of a Pediatric Oncology Program in Guatemala. In October 1995 a teaching program for nurses and physicians to increase awareness and understanding of childhood cancer was conducted in Guatemala. A computer and appropriate software for data management was placed in Guatemala and the physicians introduced to prospective investigation data management techniques. Eventually a tumor registry is planned with attendant data collection and analysis. A prospective study of the most common childhood cancer, acute lymphoblastic leukemia, has been initiated. A standardized treatment protocol has been designed in collaboration with the Guatemalan oncologists. A total of 300 children are expected to be treated in the first 3 years of the study. Basic and clinical studies are also planned for retinoblastoma. A private foundation, "A Tomorrow for Children: GUATEMALA" was established and will obtain the chemotherapy. A linear accelerator has been donated by the foundation with the condition that all children in Guatemala regardless of diagnosis or ability to pay, will be treated for free. The ultimate goals of the project are to create a multidisciplinary Pediatric Oncology Program centralized in one Guatemalan medical institution in conjunction with a children's cancer study group to collaborate with other countries in Central America in the treatment of childhood cancer.

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MONZA'S SCHOOL OF PEDIATRIC HEMATO-ONCOLOGY FOR COUNTRIES WITH LIMITED RESOURCES.

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In the last years there has been a growing interest to develop pediatric hemato-oncology services in low-income countries. In this context the pediatric hemato-oncology center of Monza has established a twinning program with "La Mascota" Hospital of Managua since 10 years. Thanks to this program there is now a service which can diagnose and treat all children with malignant diseases in Nicaragua. According to our experience twinning programs are very productive approach and should thus be encouraged and expanded. At the present time there is however also a need to define methodologies adequate to aggregate different centers and to foster cooperative researh activities in homogeneous geographical areas of low-income countries. In the last years in Italy there has been a growing consensus to the abovementioned initiative which has allowed to raise resources to expand the perspectives of the cooperation in this field and to institute the "Monza's School of Pediatric Hematology-Oncology for countries with limited resources" with the following objectives: to update technical knowledge of pediatric hemato-oncologists; to improve their methodological know-how, specifically with respect to the critical assessment of existing and competing strategies and techniques of intervention; to favour the creation of collaborative networks, capable not only of delivering a better care, but also of documenting and evaluating it epidemiologically; to promote the development of situations of care with a high degree of integration between the *medical, psychological, organizational aspects. The overall approach will be guided* by the criteria of appropriate-essential technology transfer. Representatives of some Spanish speaking countries of Central and South America have been selected to partecipate in the first course (19-28 September, 1996) whose objectives will include also the formulation and adoption of cooperative clinical epidemiological research projects. The impact of the course will be evaluated with appropriate methodology. Other courses will be organized yearly on other hemato-oncology issues. The School will also provide grants for residential training and medical literature updating activities, with the aim to contribute to a more homogeneous development of pediatric oncology.

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MYELODYSPLASTIC SYNDROME IN CHILDREN: ACCENT ON DIAGNOSIS AND TREATMENT.

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14 patients(pts) with primary acquired MDS were admitted to our hospital in 1984-1996; aged 3 to 15 yr.,9 boys,5 girls,duration of disease was 3-12mo. According to FAB classification there were RA - 4, RAEB - 7, RAEB(t)-2, CMML- 1.There were difficulties with diagnosis in 6 pts. Severe cytopenic syndrome combined with bone marrow hypoplasia (fat > 80%) was in 2pts.The diagnosis "Aplastic anemia" and splenectomy took place.Myeloid cells in spleen have been defined in one pt, who died 5 mo later from hemorragic syndrome, with no progression of leukemic process. In second case acute monocytic leukemia was diagnosed 5 mo after splenectomy and being reason of death. Next pt had severe trombocytopenia, mild leukopenia, bone marrow hypoplasia, and micromegakariocytes. This pt is alive without any treatment.Unusual symptoms like antibodies to erythrocytes and lymphocytes with high level of Ig G in 1 pt, reticulocytosis more 100 %o in 2 pts, were not typical for classical MDS and complicated diagnosis. The treatment included glucocorticoids (8 pts),low doses of cytozar(4, with interferon-alpha - 3), red cell transfusion only (2),no treatment(1).After 8-25 mo of the disease there were RA-4 (alive), RAEB - 2(1 ex), RAEB(t) - 9(8ex),CMML - 1(ex).The mean lifespan was 18 mo.More typical variant of MDS in our group was RAEB with evolution to RAEB(t), low effect of treatment and high mortality.

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6 PATIENTS WITH MYELODYSPLASTIC SYNDROME IN CHILDREN FROM KIEV REGION (UKRAINE)

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From April 1994 till April 1995, 6 children (3 - 16 years, median 9 years) with myelodysplastic syndrome (MDS) were registered in the Pediatric Oncohaematologic Department of the Kiev Regional Hospital. 4 pats had peripheral pancytopenia, 1 anaemia and neutropenia, and 1 initial anaemia followed by blastaemia. In 2 pats with BM-hypercellularity diagnosis of MDS based on FAB-criteria (RA and RAEBT). 4 pats with BM-hypocellularity showed histologically prominent myelofibrosis with fibroblastic reaction, narrowing of marrow sinuses and markedly decreased cellularity. Cytogenetic analysis in 5 pats revealed: del(5)(q33)/-4n in a pat with RA; t(9;22)(q31;q11)/-4n in a pat with RAEBT; inv(3) (q21q26) in a child with RA followed after 3 months by RAEB (but our pat had amegakariocytosis and severe thrombocytopenia in contrast to elsewhere described cases with this cytogenetic anomaly); in one child initial hypodiploidy 35-38,XY-1,-13,-18[cp3] changed to karyotype 46,XY with t(6;11) (q27;q23) which was detected in BM-blasts as in peripheral lymphocytes under the transformation from RAEBT to AML M4 after 5 months; in 1 pat with RA transformed to RAEB after 9 months, the karyotype was normal (46,XX). 2 pats (RA transformed to RAEB, and RAEBT transformed to AML) died because of septic shock after intensive chemotherapy, 1 pat with RA died due to interstitial pneumonia after 4 months of corticosteroid treatment. 2 children are lost to follow-up, and 1 girl with RA and 5q- has received symptomatic therapy and been in stable condition for 10 months. According to data in the literature most of the cytogenetic abnormalities and histologic pictures in our pats are markers of induced MDS (caused particulary by ionizing radiation). Thus, these findings in patients from the Region of the Tchernobyl catastrophe could be a direct result of this event.

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ADULT VARIETY OF CHRONIC MYELOGENOUS LEUKEMIA IN CHILDREN - REPORT OF 47 CASES FROM A SINGLE INSTITUTION

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A total of 47 children with adult form of chronic myelogenous leukemia (ACML) between the ages of 3.5 and 14 years were seen over a period of 16 years at All India Institute of Medical Sciences. Except 3 all were above 5 years of age with a striking male predominance (male:female=36:11). Thirty eight (80%) patients presented in chronic phase, 4 in accelerated phase and 5 in blast crisis. Pheladelphia chromosome was positive in 88% of cases. LAP scoring was low in all 10 patients in whom it was done. Splenomegaly was observed in all while hepatomegaly was seen in 66% of cases. Massive splenomegaly (>20cm) was present in 25% cases. Sixty-nine per cent patients presented with WBC count over 100,000/mm^3. Out of 47 patients 10 refused for therapy. Twenty four children were treated with busulphan therapy. Eight patients are still alive and on follow up between 10-156 months (mean 44 months) from diagnosis. Twenty four patients died between 3-56 months after diagnosis (21 blast crisis, 2 severe bone marrow aplasia and 1 splenic rupture). The mean survival was significantly low (25.6 months) however, interestingly 2 children are still alive in chronic phase with intermitent busulphan therapy for 11 and 13 years respectively. Presence of significant hepatomegaly, severe anemia, thrombocytopenia and bone marrow blastosis>5% at diagnosis were associated with a short survival.

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TREATMENT OF CHILDREN WITH B-NHL ACCORDING TO A MODIFIED BFM-90 PROTOCOL IN ST. PETERSBURG

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From Feb. 92 to April 95, 25 newly diagnosed children with B-NHL (22 boys, 3 girls) aged 3 - 14 ys were allocated to a modified B-NHL BFM-protocol. The main modifications of the original BFM-90-protocol (A. Reiter, Hannover) are: (1) MTX dosage was reduced from 5 g to 1 g/m^2 (24 h infusion), (2) i.th. applications were given only once in all chemotherapy blocks, but with double dose, instead of 2 injections in blocks AA and BB or instead of intraventricular chemotherapy in blocks AAz and BBz. Patients were allocated to the risk groups (RG) according to the criteria of the original protocol: 2 pats in RG 2, 23 in RG 3. The primary tumor was located in the abdomen in 22 pats, at the neck in 2, in the nasopharynx in 1. The diagnoses were confirmed morphologically in all cases. - Results: 2 of the 25 pats were non-responders, 1 pat died early due to toxic complications. 22 pats achieved complete remission. As of 1.2.96, 2 pats had suffered relapse and died subsequently. 1 pat died in CR due to septicemia. 2 pats were lost to follow-up. The projected event-free survival and overall survival at 47 months (Kaplan-Meier estimate) is 76 % (SD 9 %). - Complications: All pats developed neutropenia. Neutropenic enterocolitis (NE) was the most common and dangerous complication and occured in 17 pats. 1 pat developed peritonitis due to bowel perforation. Infections of the urinary tract were observed in 17, pneumonia in 4 pats, septicimia in 1 (pat died). The most frequent infecting pathogens were Staph. epid. (23 %), Cand. alb. (19 %), Strept. virit. (15 %), E. coli. (14 %), Enteroc. (12 %), Neiss. (10 %), vv. Herpes (6 %), Proteus (1 %). 16 cases of NE were treated without operation, 1 of these pats died. 1 pat with intestinal perforation underwent laparotomy and colostomy (survived). - Conclusion: The results obtained with the modified B-NHL-BFM-protocol are clearly superior to the historical control of our group. The toxicity of chemotherapy is high, but can be kept within tolerable limits.

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CHILDHOOD HODGKIN'S DISEASE IN CASABLANCA / MOROCCO

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The purpose of this retrospective study is to give an overview of childhood Hodgkin's disease (CHD) i.e. aged 15 years or younger, treated in Casablanca. All cases of CHD proven by biopsy seen between 1980 and 1994 are included. Staging procedure included clinical history, physical examination, chest x-ray, ultrasound of abdomen , lymphangiography or CT scan when possible, and bone marrow biopsy. Exploratory laparotomy is not used. From 1980 to 1981 patients are treated according to MOPP combination and since then MOPP/ABVD or ABV combination is used. Extended field radiotherapy is used from 1980 to 1988 and involved field afterward. One hundred eighty five cases of CHD are referred to our institution. The male/female ratio is 3.3. Thirty four patients (18.3%) are 5 years old are younger. Fifty four percent of the patients presented with mediastinal mass and 57.2% presented B symptoms. Advanced stages III and IV are found in 29.7% and 31.8% respectively. Histologic subtype when mentioned is 1 in 25 cases (19.4%) , 2 in 35 cases (27.1%), 3 in 64 cases (49.6%) and 4 in 5 cases (3.9%). Hundred and six patients are eligible for treatment evaluation. In these patients, complete remission is obtained in 89% . Overall survival at 5 years is 92% and disease free survival 70%. These results show high frequency of onset of CHD at very young age and histologic subtype 3.

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CHILDHOOD HODGKIN'S DISEASE IN NICARAGUA

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Introduction: Only few clinical data about Hodgkin's disease (HD) in children are available from low-income countries. In this study we reporte the experience of the Hospital "La Mascota" of Managua, the only pediatric hemato-oncology center in Nicaragua, where children are referred from the whole country.
Patients and results: From January 1990 to December 1995, 45 patients with HD were diagnosed and classified according to the Ann Arbor system for clinical and pathological staging and to Rye Conference criteria for histology. The following data were analysed: age, sex, staging of disease, histopathological subtypes at diagnosis, primary presenting site, response to therapy, relapses, abandons.
Out of 45 patients, 33 (73%) were male and 12 (27%) were female with male/female ratio of 2.7. Mean age was 8 years (range 3 and 15 years). 26/45 (58%) were aged between 5 and 9 years. The staging at presentation was: 5 patients (11%) stage IA; 8 (18%) stage IIA; 4 (9%) IIB; 5 (11%) IIIA; 14 (31%) IIIB and 9 (20%) IV B. Histological patterns were classified as follows: 15 (33%) nodular sclerosis, 23 (51%) mixed cellularity, 4 (9%) lymphocyte prevalence and 3 (7%) lymphocyte depletion. Primary presenting site of involvment was cervical in 32 patients, abdominal in 3, inguinal in 4, axillary in 2, mediastinal in 3 and in soft tissues in 1. All patients received only combinations of chemotherapy (CT) (radiotherapy only recently has become available in Managua): stage IA and IIA 6 cycles of CT with cyclophosphamide, vincristine, procarbazine, prednisone (COPP); IIB, IIIA, IIIB, IVB 10 cycles of CT with adriamycin, bleomycin, vincristine (ABV) and COPP. 34/45 (78%) patients completed treatment and are in 1st CCR. 4/45 (11%) are still in therapy and in CR, 4 (9%) abandoned treatment and 3 patients relapsed. Of these last 3 patients 1 is in 2nd CR following CT, 1 is being treated and 1 has died.
Discussion: This data suggest that, compared with developed countries, mean age of children with HD in Nicaragua is slightly lower, whereas the male/female ratio is rather similar. There is a predominance of mixed cellularity HD, which, however, it is not associated with poor prognosis, as reported in other studies.

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QUALITY OF LIFE IN YOUNG ADULTS WHO ARE LONG-TERM SURVIVORS OF CHILDHOOD CANCER

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Introduction. With the use of combined modalities of treatment, such as surgery, radiation therapy and multiagent chemotherapy, approximately 65% of children will be cured of their disease. It has been well established that survivors of childhood cancer have a greater risk of a number of medical problems, including a second malignant neoplasm, growth retardation, infertility, hormone deficiencies, and functional impairment of some organs. Less is known about the psychosocial late effects in adult survivors of childhood cancer, and whether their psychosocial functioning is affected by adverse medical late effects. As part of a large follow-up project among adult survivors of childhood cancer, a study was done to assess their psychosocial adaptation and quality of life and explore its relationship with medical problems in this population.
Method. The patient sample was composed of 150 adult survivors of childhood cancer, age >18 years, who had been treated in our hospital in the period between 1975 and 1990, and who were free of the disease for >5 years after completing therapy. The participants completed a questionnaire which we developed, including existing standardized measures and a Dutch version of Chessler et al.'s questionnaire. It contains the following domains: demographic and medical information; educational achievement; occupational status; work and insurance discrimination; quality of life comprising physical, psychological, sexual, and social functioning; experienced health status and worries about health; life style and philosophy of life; relationship with family and friends; self-esteem and post-traumatic stress.
Results. Data-analysis is in progress. The first results of this study will be presented.
Implications for nursing and psychosocial practice. The study may contribute to further understanding of the quality of life in this population of adult survivors of childhood cancer and to clinical and research implications for pediatric oncology nursing and psychosocial practice.

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QUALITY OF LIFE OF YOUNG ADULTS IN ISRAEL WHO HAVE RECOVERED FROM CHILDHOOD CANCER

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In the year 2000, one in every one thousand young people will be a "survivor" of childhood cancer. Today more and more children recover from cancer. This study examines the quality of life of 64 young adults, aged between 18 to 35, who had childhood cancer, as compared with an appropriate control group. Quality of life was defined in the broadest sense of the word and included three priniciple areas: physical health and daily functioning; mental health, including depression, anxiety, overall satisfaction and so on; and social adjustment, including education, occupation, family status, military service and social support. The subjects were chosen from a follow-up clinic treating over 700 patients; all had been diagnosed before age 18 years and had been under care for two years with no signs of the illness. Participants received a questionnaire which also included demographic particulars and data from their medical files. The results showed that the quality of life of the survivors was similar to that of the control group, but with some striking differences in the areas of physical health, military service and social support. Mental health status was identical in both groups, including fear of recurrence of the illness or the appearance of a different form of cancer. The findings of this pilot study will be used for further documentation and research, and for applications to current treatment.

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EDUCATIONAL LIAISON PROGRAM FOR CHILDREN WITH CANCER AND CANCER SURVIVORS

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An educational liaison program at the University of California, San Francisco has been established to assist children on and off therapy with school problems. The program is directed by a nurse/teacher and description of the program is offered as a model for other medical centers. The service goals of the program are to facilitate a successful return to school following a cancer diagnosis, to minimize days absent from school during therapy, to provide pediatric oncology education of school personnel in geographic areas served by the medical center, and to secure special educational support for patients if needed. Research aims are: to monitor school success of children by an annual teacher assessment form for children on and off therapy with regard to treatment effects; to create and verify an educational risk scale for newly diagnosed patients. Approximately 80 children a year are diagnosed with cancer at this facility and 600 survivors are monitored annually. Children who have received cranial radiation and/or intrathecal chemotherapy, have a central nervous system relapse, received a bone marrow transplant or have a family history of special education are of particular concern for the educational liaison. We provide the following educational interventions: 1) School re-entry visits are scheduled for children who are newly diagnosed. The school re-entry program is conducted with a special curriculum to explain the disease and treatment of the child with cancer to their classmates and teachers. 2) If a school concern is identified for children during therapy or off therapy, the educational liaison interviews the family and child to determine a strategy for intervention if indicated. The school teacher is contacted as well as the counselor, principal or school nurse as appropriate. 3) Further school interventions may include: special education assessment and planning; education of school personnel with mailed written material or in-service workshops; identification of educational support such as tutoring services or computers. 4) An annual conference for school personnel increases awareness of educational sequelae for children with cancer. The program is intended to address the educational needs of children with cancer, to help families obtain appropriate services, to evaluate student achievement and identify and target high risk newly diagnosed children for educational intervention. Results of on-going research will be reported in the future.

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SURVIVING CHILDHOOD CANCER: LATE PSYCHOSOCIAL CONSEQUENCES FOR PATIENTS, PARENTS, AND SIBLINGS.

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Aim: Progress in medical treatment has drastically raised the rate of survival in childhood cancer. Consequently, the focus is now on the quality of this survival. Since childhood cancer affects the whole family, we investigated the late psychosocial consequences for patients, parents, and siblings.
Method: Seventy-one families with a child who had successfully terminated cancer treatment participated in the study (compliance rate: 97%). The psychosocial functioning of each family member was measured by a variety of assessment techniques, including in-depth interviews, psychological tests, illness-specific questionnaires, and clinical judgements based on observations.
Results: Patients were significant more withdrawn and showed more social problems than healthy peers. Especially boys were at risk for serious psychosocial problems. Parents reported a number of significant changes in their life resulting from the illness. They lost important values in the outlook of life, such as loss of joyfulness and loss of invulnerability. As a result mourning processes prevailed in spite of the child's survival. In addition, uncertainty and loneliness were reported. When confronted with late medical sequelae, parents reported more problems. Lingering concerns of the siblings focussed on the relationship with their parents and their position within the family. For all family members, problems did not decline over time. The general adjustment of each family member was assessed as measured by the (Child) General Assessment Scale ((C)GAS). Results are shown in the table.

	Children		Parents	
(C)GAS category	Patients (*n*=67)	Siblings (*n*=56)	Fathers (*n*=63)	Mothers (*n*=71)
Moderate to marked adjustment problems	31% (*n*=21)	9% (*n*=5)	6% (*n*=4)	11% (*n*=8)
Mild adjustment problems	16% (*n*=11)	5% (*n*=3)	24% (*n*=15)	28% (*n*=20)
No adjustment problems	52% (*n*=35)	86% (*n*=48)	70% (*n*=44)	61% (*n*=43)

Conclusion: Surviving childhood cancer has consequences for the whole family. The nature of these consequences, however, differs for patients, parents, and siblings. The complexity, seriousness, and persistent duration of these consequences underline the need for psychosocial follow-up services.

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QUALITY OF LIFE AND PSYCHOSOCIAL ADJUSTMENT OF YOUNG PATIENTS WITH MALIGNANT BONE TUMOURS AFTER TREATMENT END

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In recruiting patients for this quality-of-life study, we established the following criteria: age between 15 and 30, tumour localisation at the extremities and time since treatment end at least 1 year. Out of 110 patients, 63 were willing to participate. The assessment of quality of life was conceptually based on four measures: physical, psychological, social and functional status. Physical and functional status were evaluated by 2 physicians, for psychological assessment a series of standardized psychological tests and an interview were implemented. The majority of our patients, i.e. approximately 80%, show no or only minor psychosocial problems. They could adapt well to their new living-conditions, though strong efforts like getting used to restricted mobility, catching up with school or the change of jobs or job orientations had to be made. 71% have a loving relationship, one third of the patients older than 19 are high school or university graduates. Most of our patients are strongly attached to their families and value their support as being most important during therapy. Clinical data as well as physical or functional sequelae have no effect on psychosocial adjustment, with one exception: patients who fell ill during adolescence show significantly more problems, specially in the dimension social well-being, than patients with diagnosis in childhood or early adulthood (Anova, p=.05). Our findings suggest that survivors of bone cancer are not necessarily at risk of developing long-term emotional or social problems and are not precluded from leading active and independent lives.

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HEALTH STATUS ASSESSMENT IN YOUNG ADULT SURVIVORS OF OSTEOSARCOMA AND EWING TUMORS OF THE EXTREMITIES

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The purpose of this study was to evaluate the health outcome in a population of 57 extremity sarcoma (44 osteosarcoma and 13 Ewing tumors) survivors, observed after a median time of 8 years from diagnosis. Forty-six patients had limb-sparing surgical techniques; 11 underwent amputation. The assessment instrument was derived from the Impairment Ratings by O'Malley and consisted of six domains: obviousness of residua; interference with activities; medical attention; employability; hearing loss; and pain. Each domain was conceived as a vector so that we could define a global health score with the use of the vector sum. Compared to the O'Malley ratings, this resulted in a more accurate description of the individual's health status. - Limb-spared patients scored better in almost all domains than did the amputated. By contrast, they needed more operations and medical controls, suffered more physical pain, and had to deal with recurrent local problems of implant instability, infection, or neurapraxia. Exclusively among the male subjects, cancer chemotherapy performed before puberty accounted for a reduction of 5 cm in body height. Plotting health scores against time after tumor diagnosis showed a U-shaped curve with the poorest health in patients observed 5 to 10 years after diagnosis. This finding probably reflects a separation of the survivors into two groups during this crucial period: those who continue toward late fatal complications, and those who do not. Physical health assessment correlated poorly with the orthopedic and psychological testing results, stressing that these methods constitute three complementary approaches to understanding health-related quality of life.

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HOW VIOLENTLY CHILDREN EXPERIENCE AMPUTATIONS

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Amputations continue to be major psychological ordeals for children and parents. Five clinical examples will illustrate this. 1- After his operation, a teenager asked his doctor :l- « What did the part they removed from me weigh ? « meaning, « Was my leg not simply a piece of flesh ? ». 2- Having learnt that there was no hope, a teenager's father threw his son's prosthesis away violently, shouting : « Take it back doctor, it's your's ». He felt deprived of his parental authority and his son had become a complete stranger to him. 3- A father could neither announce his son's imminent amputation, nor give the surgeon permission to operate. A psychotherapeutic interview helped him to discover the link between the present situation and a dramatic event in his own adolescence, and to overcome his fear. 4- A teenager spoke of his leg as if it were a ghost that was haunting him. He said he was a robot and a mutant. He was losing his sense of identity. 5- A young girl expressed, in her drawing, the difficulty she was having in attaching to and integrating into her conscious and unconscious image of her body, her missing leg, even though she accepted her new physical identity. Our experience of interviews with children who underwent amputations shows that the violence of this amputation can be overcome and accepted by children and parents but their confidence must be gained from the outset, specific explanations and a psychological work up are required.

O-245

QUALITY OF LIFE AFTER BONETUMORRESECTION: A COMPARISON BETWEEN ROTATIONPLASTY, ABOVE-KNEE AMPUTATION AND HIPEXARTICULATION.

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The Van Nes-Borggreve rotation plasty is a reasonable alternative to above-knee amputation or hipexarticulation, however, at the cost of physical appearance and perhaps psychologic functioning. Quality of life (QL) was therefore assessed in patients with a rotationplasty (n=33), above-knee amputation (n=13) and hipexarticulation (n=6), using standarized QL measures. Patients were 16 years or older and more than one year post-operative. The response rate was 90% in each group. There were no systematic nor significant differences in sociodemographic characteristics, follow-up, disease stage and complication rate across the three groups.
The rotation plasty group had a better physical performance and had fewer problems related to their prosthesis. For example, 27 patients with a rotation plasty ride a bicycle and 32 out of 33 patients are active in sports. The three patient groups did not differ significantly nor systematicallyy with respect to reported levels of self-esteem, vitality, social functioning, emotional functioning and mental health. Few patients reported to have a severely impaired body image (4,2 and 2, respectively) and sexual functioning (2,1,1, respectively).
Conclusion: a rotation plasty after tumorresection gives good functional results and the psychosocial outcome is satisfactory and comparable with above-knee amputation and hipexarticulation.

O-246

LIFE QUALITY AND FUNCTION AFTER LIMB SALVAGE OR AMPUTATION IN PATIENTS WITH OSTEO- OR EWING'S SARCOMA

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Although limb salvage is established as a standard treatment of malignant bone tumours, only a few articles deal with psychological and functional outcome after limb salvage or amputation.
Out of 110 patients operated between 1972 and 1993, 63 were examined after a mean follow up of 96 months (range 24-228). The mean age at operation was 15 years (range 1-25). 35 patients were male, 28 female. The histopathological examination revealed 48 osteosarcomas and 15 Ewing's sarcomas. According to the type of reconstruction three groups were separated; A: biological reconstruction, B: endoprosthesis and C: amputation and rotation plasty. Psychological criteria included emotional and social well-being, subjective efficiency, partnership and sexuality. Functional evaluation was performed according to the Enneking criteria.
Function was superior in group A: 29 points (range 22-30) and group B: 27 points (range 11-30), but inferior in group C: 23 points (range 15-30).
There was a low correlation between Enneking score and a) psychological well-being (r=0.03) b) social well-being (r=0.08) c) partnership (r=0.28) and d) subjective efficiency (r=0.03).
The psychic deficiency of 20% is not higher than in the norm population. The low correlation between the functional results and psychological parameters corresponded with poor covariation of subjective and objective life quality. High subjective life quality was found in the presence of poor living conditions as well as contrary. This discrepancy probably can be explained by different the individual cognitive valence of patients.

O-247

PARENTS' COPING WITH THEIR CHILD'S CANCER AND THE PARENTS' SATISFACTION WITH THE INSTITUTION: A RETROSPECTIVE STUDY

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Our study is dealing with the parents' adaptation to the child's cancer and their satisfaction with the institution. The sample consisted of 632 mothers and fathers of patients who had been treated during the last 25 years on the oncology units of the St. Anna Kinderspital in Vienna and who survived the illness. By using a specificly designed survey parents were asked how they coped with their child's illness and how satisfied they were in the hospital. Confirmatory factor analytic procedures employed in the present study supported the distiction in five active and five passive strategies (Heim,1983) of coping with cancer. By setting up a model following these factors, the influence of parents' coping strategies on their satisfaction with the hospital was proved. Our results also emphasize that information about the child's illness given to parents is most important for their compliance and satisfaction in the hospital. No correlation was found between a specific diagnosis of the child and the parents' coping style or their anxieties in the present.
The major conclusion of these findings is that active and passive coping styles have an impact on how satisfied parents are in the hospital and with the information given to them. In addition, the results showed that a „Damocles syndrom" (Koocher & O'Malley, 1985) exists also for parents which confirms the importance of psychological support for the family after the child's illness.

O-248

HOSPITAL, DIFFERENTLY

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To favour a life of quality to a child during his illness, our concept of care intend to undeceive the world of the hospital, to improve the entire care (the charge) of the child (whatever his age), to favour the familiar ties by doing of the family some partners of care.As a whole, we have to take into account the rythm of life for each child, it means their inconveniences, their activities, their studies, their familiar imperatives. Indeed, to preserve the child and to keep him in a regular life, our policy is both to reduce the time in the hospital and to favour the fact to be hospitalized more during the day and this as short as possible. the National Education became our partner and now we can appreciate the presence of 3 teachers who give a schooling once in the hospital and once in the house. To improve the stay of a child, differents rooms have been designed (conception, choice for the colors, furnitures, decoration, specific frame, a room for games, a outdoor *parc, a reception room). The hospital's* world needs to be open to the outdoor world. Indeed the contact with the outdoor world is essential for a child, that's why we have created several daily activities. Educators and staff suggest each day differents activities like painting, pottery, reading, computer class, games (inside and outside : bicycle, playing in sand). Even better we have the intervention of clowns, readers, actors, musicians, Mister carnival and Santa Claus. At the present time, the Art class makes a mural fresco. All those additionnal elements of the medical charge enable for a child and his family to fight against the sickness, to confront the treatment, to keep a permanent tie with the outdoor world by favouring the return to a regular way of life.

O-249

AN INNOVATIVE OUTPATIENT ARTICULATION PROGRAM FOR HOSPITAL-BASED PEDIATRIC ONCOLOGY NURSES

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The increased diversity and level of sophistication which now exists in the care of children with cancer mandates a concomitant level of knowledge, training and skill on the part of those who implement the care. The decrease in required hospital admissions and inpatient census rates provides fewer opportunities for novice oncology nurses to develop expertise in all aspects of pediatric oncology care. In some cases, inpatient oncology nurses may gain little or no experience in disease areas such as Hodgkin's disease or Wilms' tumor, where hospitalization rarely occurs, except at the time of diagnosis. In an effort to provide education and mentoring to novice pediatric oncology nursing staff, an innovative outpatient articulation program was developed at our institution. As part of the structured hospital orientation, pediatric oncology nurses spend scheduled days in the outpatient setting working with attending-level physicians, the pediatric oncology nurse practitioner and experienced pediatric oncology nursing staff. Instruction, utilizing a cognitive/developmental curriculum design, focuses on specific intended learning outcomes necessary to the practice area. Instruction progresses through stages of practice development according to the Benner Novice-to-Expert Model. The program allows nurses to gain valuable cumulative experience in the care of many patients receiving diverse therapies. Nurses also receive individualized education regarding clinical research in pediatric oncology, both on a local and national level. Evaluations reveal that staff perceive they are better resources for patients and families after the experience. The majority have elected to work part-time in the outpatient setting in addition to their hospital position. Families consistently praise the continuity of care which results.

O-250

MEASUREMENT OF PAIN IN CHILDREN WITH CANCER: WHAT DOES THE NURSE REPORT?

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Introduction. Children with cancer often experience pain. This can be caused by the illness itself, the treatment or its complications, or by invasive diagnostic procedures. Nurses play an important role in the assessment of pain. It is part of the nurse's domain, and is one of the nursing diagnoses accepted by the NANDA. The assessment and the nurse's findings as reported in the child's record are necessary for the adequate treatment of pain. In order to improve the assessment of pain experienced by children with cancer, the nurses on the pediatric oncology ward of the Emma Kinderziekenhuis AMC use a pain anamnesis and a pain questionnaire. They also assess the child's own perception of his or her pain twice a day using the Oucher or VAS (as appropriate to the child's age).
Method. The purpose of this study was to discover whether the number and quality of reports about pain in nurses' daily records improved after the introduction of the pain anamnesis, the pain questionnaire and the children's own reports of their pain. We studied these records using a *Dutch version* of 'Camp's Chart Survey Information Form', which was developed to classify the nurses' pain registration in 11 categories. We used nurses' records of children with cancer aged 4-18 years. In this retrospective nursing intervention study, we used a non-equivalent three group design. First we studied 60 records before the introduction of the pain anamnesis and the pain questionnaire. Two months after these instruments were introduced, we studied another 60 records. Nurses then began assessing the children's perception of pain twice a day using the Oucher or VAS. Two months after that we studied a further 60 records.
Results. If the interventions mentioned above improve the observation and assessment of pain, the literature would lead us to expect a corresponding improvement in the amount and quality of pain-registration in the nurses' daily records. The results of these study will be presented at the conference.
Implications for nursing practice. The experiences of nurses using the pain instruments and the influence of these instruments on the quantity and quality of nurses' reports about pain in children with cancer will be discussed during this presentation.

O-251

Mouthcare - ritualistic practice reconsidered

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Oral complications are a common problem associated with all modalities of cancer treatment (Beck, 1992). There is a general agreement that oral care cannot prevent the damage caused by cancer treatment but it may prevent infections that cause further damage to the oral tissue.
The prevention and management of oral complications remains a major challenge to health care providers within oncology (Beck, 1992). However, there is some concern that current oral care procedures appear to be largely based on tradition, anecdote or subjective evaluation (Holmes, 1991). Furthermore, scientific rationale for oral hygiene measures are seldom mentioned in the nursing literature and on occasions conflicting or contradictory suggestions are made (Daeffler, 1980). These problems have been identified within the unit where the use of an oral assessment guide (Eilers et al, 1988) is well established facilitating skills in assessing and evaluating care given. Reflecting on our clinical practice it was identified that a proportion of children were receiving inappropriate mouthcare; the same mouthcare regime was being prescribed for nearly all children irrespective of their chemotherapy. To ensure that all children undergoing chemotherapy received appropriate and standardized mouthcare that was based on scientific rationale a working party was established. The result of their activities was the production of a mouthcare protocol with accompanying flowchart. Action research as a change of strategy (Webb, 1990) was the approach taken by the working party; this was a cyclical process that included diagnosing, action planning, action taking, evaluating and identifying general findings (Susman and Everard, 1978).

O-252

HIGH-DOSE METHOTREXATE: MULTIDISCIPLINARY SUPPORT AND AMBULATORIAL CARE

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High-dose methotrexate (HD-MTX) regimen for the treatment of osteogenic sarcoma consists of 12 g/m^2 over four hours infusion and it is widely used. Patients must be well hydrated, urine must be alkalinized, serum MTX levels must be monitored carefully after the end of the infusion followed by leucovorin rescue to prevent severe toxicity. The development of an ambulatorial HD-MTX infusion program in our outpatient clinic was necessary and based on these principles. In order to have close control of those parameters at outpatient basis, we designed an special instrument called orientation sheet (OS). Through the OS, the process of teaching, monitoring and the evaluation of these patients were feasible and the family members were also closely involved with the treatment. From October/91 to February/96, 32 patients were enrolled, receiveing 172 cycles of HD-MTX and being guided through the OS at each time. From all patients, six (6) presented mild mucositis, five (5), moderate mucositis without any food intake alteration, three (3) patients presented severe mucositis and two (2) of these had to be hospitalized. We conclude that with an appropriate support care where the economical, social and cultural aspects are taken into consideration by the multidisciplinary team, the HD-MTX ambulatorial infusion is safe and play an invaluable role in the treatment of these patients and their families.

O-253

ADMINISTRATION OF HIGH DOSE METHOTREXATE (12 G/M^2) AND MANAGEMENT OF TOXICITY IN THE OUTPATIENT SETTING

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High dose methotrexate (HDMTX) can be safely managed in an outpatient setting for the pediatric patient (pt) with osteosarcoma. Approximately 200 doses/year in pts aged 5 to 30 are successfully managed in the Pediatric Day Hospital (PDH). Extensive patient/family teaching and meticulous nursing care are required to complete the 4-day therapy. Hospital admissions are few. Patient/family education includes (1) one-on-one teaching session with the Pediatric Nurse Practitioner and (2) review of the HDMTX resources. Goals for the first 24 hours of therapy are to establish and maintain an alkaline urinary output, administer the HDMTX, initiate leucovorin rescue, achieve an output of 1500 ml/m^2 and prevent nausea/vomiting, all of which are assessed after 24 hours along with the serum methotrexate (MTX) level and renal function. Vigorous oral and intravenous (IV) hydration and urine alkalinization are initiated to reduce the potential common side effects of renal toxicity and impaired clearance of MTX, oropharyngeal mucositis, cutaneous erythema and desquamation, and pancytopenia. This regimen of 10-hour therapy in PDH, daily monitoring of serum MTX levels, overnight hydration and alkalinization at home via an ambulatory infusion pump (AIP) and vigilant follow-up care continues from Day 2 through Day 4 until the serum MTX level is nontoxic (<100 nM/L). Renal and serum MTX toxicitiy are treated with increased oral/IV hydration, and the dose of oral/IV leucovorin rescue is adjusted according to the PDH MTX toxicity grading tool when the serum MTX level is <50,000 nM/L (nontoxic at 24 hours <10,000 nM/L). Pts with serum MTX levels >50,000 nM/L are treated with IV leucovorin rescue via an AIP. Pts who fail to achieve a nontoxic MTX level on Day 4 continue with leucovorin rescue and oral/IV hydration until the level is nontoxic. This aggressive treatment modality allows outpatient care to continue even if MTX toxicity occurs, thus limiting costly hospital admissions and normalizing family life.

O-254

AN EVALUATION OF PARENT-ADMINISTERED HOME INTRAVENOUS DRUG THERAPY

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In many childhood cancer centres, parents undertake the routine management of their child's central venous catheter. In some units, this extends to administering intravenous drugs to their child at home. A prospective study of parent-administered home intravenous therapy in one centre over one year was conducted, to ascertain the safety and desirability of this development. Clinical data from hospital casenotes, and parents' self-report via anonymous postal questionnaires was collected. A total of 469 days of home treatment were given during the study period. Three types of treatment were involved; chemotherapy (19 courses/129 days), antiemetic therapy (17 courses/78 days), antibiotics (59 courses/262 days). This represents a considerable saving in in-patient days and outpatient visits for families and staff. Few clinical problems were encountered, occurring most commonly in neutropaenic patients; it is felt that these events were not related to the home treatment policy. Parent's views of home therapy were positive; they welcomed the involvement in their child's treatment, felt well supported in the home, and reported benefits to themselves, the sick child and the family. We conclude that parental administration of intravenous treatment at home is a safe and desirable alternative to hospital admission for some patient groups. Continued vigilance, and prompt action by parents is required if children become unwell; the role of the nurse in educating parents is stressed.

O-255

HOME NURSING FOR PEDIATRIC ONCOLOGIC PATIENTS - PRESENTATION OF A PROJECT AT THE ST. ANNA CHILDREN'S HOSPITAL

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In October 1995 we started the project 'Home Nursing for Pediatric Oncologic Patients'. The Goals of this project are

- Help and support for families who want to accompany their dying child at home
- Reduction of out-patients appointments between therapies
- Help for the sick child's siblings
- Improvement of communication between all parties involved (patient, family, hospital, family doctors)
- Advising and teaching the parents in special nursing care
- Bereavement counseling
- Cooperation with the oncologic department of the St. Anna Children's Hospital, the physiotherapists, the psycosocial team and the hospice.

In this project two full-time nurses are employed at the moment, each of them having worked more than five years in the pediatric oncologic field.

O-256

THE ORGANISATION OF HOME BASED PAEDIATRIC ONCOLOGY PALLIATIVE CARE IN THE NORTH OF ENGLAND

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Despite advances in the treatment of childhood cancer, one third of all children will die, the majority as a result of progressive disease. In our experience children and families express the wish to be cared for at home during the palliative phase of their illness. This requires an intensive coordinated approach to ensure that the needs of both patients and families are met. During 1985/86 the Paediatric Oncology Unit in Newcastle upon Tyne identified limitations in the provision of support available to families caring for children with cancer. As a result, a Paediatric Oncology Outreach Nursing Team was established in October 1987 with an aim to facilitate optimal home based care by working in partnership with families, the tertiary centre, and the child's local health care services. By developing a collaborative approach to the provision of support it is possible to empower families and increase their feelings of confidence and control whilst promoting their role as primary care givers. From October 1987 to January 1996 135 children have died from progressive disease. Since the establishment of the Paediatric Oncology Outreach Nursing Team there has been an increasing number of children who have been cared for and died in their preferred home environment (36% 1986, 90% 1995). This presentation will outline the organisation of home based Paediatric Oncology palliative services in the North of England. The extent of care and complex array of symptoms managed within the home environment will also be described.

O-257

NURSING CARE AND PEDIATRIC ONCOHEMATOLOGY IN BULGARIA

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The first school for nurses in Bulgaria was established in 1900. Nowadays there are 6 medical schools for nurses. The teaching course takes 2 years and half year practice in a hospital.

Since 1995 a high nursing shcool is functioning. At the High Medical Institutes in Sofia, Plovdiv and Varna there are Pediatric Oncohematology Clinics. I am working at the Pediatric Oncohematologic Clinic in Varna. Our clinic has 31 beds North-Easting Bulgaria. The doctors are also teaching students at the Medical University. There is a Day Care Unit with 6 beds. We have a school for the hospitalized children and a tutor. Since 1993 a parent's and doctor's Society against Cancer is established.

The organisation of the nursing care for the best management of the sick children depends also on the local health and social care system.

From my first participation in the SIOP Meeting in Paris I understood that the organisation of the nursing care in Bulgaria is not very familiar to you. That's why I am taking the opportunity to inform you about the nursing care in Bulgaria and, especially, at the Pediatric Oncohematology Clinics.

O-258

CHILD AND PARENT SUPPORT IN PATIENTS FROM DIFFERING SOCIO-ECONOMIC BACKGROUNDS

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Changing demands have required the staff of the Paediatric Haematology and Oncology Unit of the Johannesburg Hospital to evaluate the appropriateness of the psycho-social support programme for patients and parents. A multidisciplinary team employs a holistic approach with psychosocial support systems developed along the lines seen in Europe and North America. Current referral patterns have resulted in a significant increase in the number of rural patients being admitted for therapy, resulting in a patient and parent population which is a mix of people from first world and third world backgrounds. Our finding is that the needs of the two groups differs. The level of education of the rural parents is poor and family income low. In many instances the child comes from a single-parent family and prior to diagnosis may have been staying with an elderly care-giver and not the parent. It is not uncommon for the child to be transferred to the referral centre by ambulance, unaccompanied by the parents. On discharge the patient returns to a home with overcrowding, lack of sanitation, electricity and no telephone. Our previous philosophy of treating wherever possible on an outpatient basis is no longer practical, with the need for prolonged hospital stays and increased bed occupancy. An increase in the number of patients with poor compliance has been noted. There is a lack of understanding of the need for continued therapy once the child is in remission. Problems of communication are encountered not only because of language differences but also because of cultural differences which, for example, require the staff to be aware and sensitive to differing concepts of death and consequently preparation for death. While the problems enumerated above are by no means unique, they pose a challenge to the staff who are required to deal with patients from such differing backgrounds.

O-259

JOINT LODGING IN PEDIATRIC ONCOLOGY

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JOINT LODGING IN PEDIATRIC ONCOLOGY

At the Hospital Erasto Gaertner a Joint Lodging System is provided, that is, the mother or another relative of the female sex stays with the child 24 hours a day, while the child is hospitalized. The mother's stay is proposed because she is considered a member of the multidisciplinary team assisting the child. Such assistance consists of the work of physicians, nurses, psychologits, nutricionists, physical therapists, social workers, all intent to provide for the needs of the patient and his/her family. When the patient comes to the institution, he/she undergoes a screening by a doctor. In the case of oncology patients, a file will be opened to record all procedures, both as an out-patient and as an in-patient, carried out during that patient's treatment. The second step will be an appoitment with the doctor, followed by an interview with the nurse and the psychologist, to explain how the work is carried out and clarify any doubts that may have lingered on after the appointment with the doctor. The Pediatrics Departament of the Erasto Gaertner Hospital has four wards with 5 beds and 6 bedrooms for in-patients. It also has an out-patient unit, an out-patient chemotherapy unit, and a classroom and teacher for school-age children. By providing this kind of assistance we try to interact with the patient and the family, offering the opportunity and possibility of a cure.

O-260

PROVIDING INTENSIVE THERAPY FOR GEOGRAPHICALLY ISOLATED PATIENTS.

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We have looked at the difficulties associated with offering contemporary frontline therapy to children with cancer from families who are geographically remote. The New Children's Hospital has 350 beds and is the principal referral hospital for children in NSW. The 20 bedded Oncology Unit and attached outpatient clinic has treated over 1700 children with cancer. Over 1000 of these have come from metropolitan Sydney and 500 from country areas of NSW. Two hundred have come from other states of Australia or overseas. Numerous difficulties arise for families from these distant areas. Some of the towns in NSW are 700km from Sydney. Intensive inpatient therapy is particularly hard on the families, as it involves long separation from other family members, local support networks and their workplace.

As an example of these problems, we describe in detail a family from Griffith, which is 600km from Sydney. This is 7-8 h drive from Sydney, and 3h drive from their nearest paediatrician. Their 4 year old daughter was diagnosed with Ewing's sarcoma in 1993. For much of the next year they travelled to Sydney for chemotherapy and surgery. Following relapse, they have spent most of the last year in Sydney, for further chemotherapy and a double autologous bone marrow transplant. The Hospital's Ronald McDonald House (the first established outside North America in 1975) has provided accommodation for both parents, while the other three sons remain at home, visiting in School holidays.

Working with families to overcome the difficulties associated with providing such treatment so far from home is an important component of the holistic care provided within the Oncology Unit.

O-261

ALTERNATIVE TREATMENT INITIATED BY PARENTS OF CHILDREN WITH CANCER; A STUDY

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The study consists of findings from a questionnaire, and interviews of 57 parents of children with cancer, 24 of which were parents of children who had died.
Findings showed that 36% of the parents of children undergoing treatment, and 63% of parent of children who had died, reported having used alternative treatment of various forms. The most common of these were herbs, vitamin-diets, mineral-diets, religious varieties, homeopathic medicine and healing - in that order.
Parents actually had little faith in the healing power of alt.treatm., but believe in the supplementary strengthening effect. They also find themselves under considerable pressure, both from within themselves and from the outside world. In the first phase, they tell of a need to do something actively for their own child. In the event of a terminal phase, they have a desperate need to "try everything", partly induced by pressure from friends and relations. Parents express the need for proper information in this bewildering field, given at en early stage of treatment, in order to be prepared for these reactions.
The study proposes that health personell must meet this need for information in order to keep an open dialog with parents at all times.

O-262

RETROSPECTIVE ANALYSIS OF THE QUALITY OF PATIENT AND FAMILY CARE IN PEDIATRIC ONCOLOGY

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High quality of care of pediatric oncology patients includes medical and psychosocial care. One quality-indicator is the retrospective assessment of subjective anonymous judgements of parents concerning important aspects of medicine, nursing, psychological and social care.

Method: In a retrospective study we asked parents whose children had been treated with chemotherapy between 1990 and 1993 for their subjective judgements. Questions included aspects of chemotherapy, familial stress as well as the hospital situation. We asked for familial content with medical, nursing, and psychosocial care. We assessed the satisfaction of the parents using a questionnaire with a 4 point ratingscala (0-3) and included parameters of length and course of treatment, risk group, relapse or death for data analysis.

Results: 106 (84.8%) out of 125 families responded. The total "index of parental satisfaction" was 2.2. Families were content with the care of physicians (2.2), nurses (2.3), teacher (2.3), social worker (2.2), and psychologist (2.2). Content was not influenced for most of the parameters. The over all "index of stress" was 1.4. Stress was highest in families who stayed more than 14 days per chemotherapy phase on the ward, parents of children with high risk, with many complications, with relapse, and of children who died.

Conclusion: The retrospective assessment showed that the families in general are content with the medical and psychosocial treatment they received. There is, however, a need for special care for families who experience specific kinds of stress. We can improve familial content and reduce stress, if we define more precicely our standards of care and utilize them in routine medical and psychosocial work.

O-263

ART AND MUSIC THERAPY WITH DANGEROUSLY ILL CHILDREN AND JUVENILES

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The paper lays stress on two essential main thoughts. Firstly, work with non- verbal media helps the process of communication. Considering the seriousness of the disease and of its treatment this is necessary in an exceptional degree in order to meet the young patients' phantasies and fears.

Secondly , this work increases creative powers in an ego-supporting sense. This often opens new perspectives and is a counterbalance to the long duration of the treatment.

The discourse will be illustrated by picture and sound material.

O-264

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL ALTERATIONS IN HEPATOBLASTOMA UNDER CHEMOTHERAPY

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Chemotherapy is one of the major parts in treatment of hepatoblastoma. In many cases the resection of tumor is done after several cycles of cytotoxic drugs. The aim of the presented study was to investigate whether some of the regressive changes seen in these tumors are specifically related to the chemotherapy, and whether prognostically important features are found after chemotherapy.

Biopsy specimens before and the resected tumor after chemotherapy were available of 30 patients treated in the German Liver Tumor Study HB 89. These tissue sections were investigated in regard to amount and type of viable tumor tissue and type and amount of regressive changes. In addition, several immunohistochemical studies were done including antibodies against cytokeratins, neural markers, and p-glycoprotein, using the APAAP method on paraffin embedded tissue.

In general the amount of viable tumor tissue was reduced in most cases, ranging from very little remaining tumor island to very discrete tumor cell reduction. The amount of fetal type of hepatoblastoma was increased after chemotherapy as compared to biopsies before chemotherapy. Nevertheless, a complete shift from pure embryonal type to pure fetal type was not seen. Mesenchymal elements as osteoid islands are more often seen after treatment as in initial biopsies. Only in a few cases the osteoid was seen only in the resected tumor after chemotherapy. Squamous epithelial metaplasia and tumor cell with chondroid differentiation were only found after chemotherapy.

In conclusion we found certain changes of histology after initial treatment with cytotoxic drugs. It is not clear whether the increase of fetal tumor tissue is just a selection of tumor cells with poor response to treatment or a true 'maturation'. The increase of osteoid might be due to shrinkage of tumor as result of destruction of epithelial components.

O-265

THE HISTOGENESIS OF HEPATOBLASTOMA. ULTRASTRUCTURAL AND IMMUNOELECTRON-MICROSCOPICAL EVIDENCE OF DERIVATION FROM A PLURIPOTENT STEM CELL.

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The wide range of epithelial and mesenchymal lines of differentiation seen in hepatoblastoma suggests that this tumour derives from a pluripotent stem cell. To test this hypothesis, examples of this tumour were investigated for the presence of cells resembling the oval cells of rodents that are thought to be closely related to hepatic stem cells. The tumours were investigated by electron microscopy, immunoelectron microscopy, and immunohistochemical staining for various cytokeratins (CK) and albumin. Small epithelial cells (SEC) corresponding to the oval cells of the rat were found. The SEC were characterized by their small size, round to oval shape, intercellular junctions and tonofilament bundles. Immunoelectron microscopy showed that they express both albumin and CK-7. They were found in small numbers in fetal hepatoblastoma and in moderate numbers in embryonal hepatoblastoma. In small cell hepatoblastoma, nearly all the tumour cells exhibited SEC-like features. Thus, cells with the features of hepatic stem cells are detectable in hepatoblastoma. The dependence of their numbers on the subtype is consistent with the theory that embryonal and, with further differentiation, fetal tumour cells derive from precursor small cells. The findings support the hypothesis that hepatoblastoma derives from a pluripotent, probably entodermal or even less committed, stem cell.

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THE HGF RECEPTOR (c-MET) AND CANCER OF THE LIVER

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The HGF receptor family includes tyrosine kinases encoded by three oncogenes: *MET, SEA* and *RON*. The members of this gene family share a unique functional feature: they mediate cell dissociation and motility ("scattering") in physiological conditions, and invasiveness in their activated versions. The *MET, RON* and *SEA* receptors display a signal transduction distinctive behaviour. Unlike conventional growth factor receptors, their cytoplasmatic tails contain a multifunctional docking site. Upon autophosphorylation, this squence binds and simultaneously activates multiple SH2-containing transducers, including Ras and PI 3-kinase. A deregulated activation of this "supersite" triggers a dramatic pleiotropic signal, responsible for invasive cell growth in carcinomas derived from hepatocytes and other epithelial cells.

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THE HEPATOCYTE GROWTH FACTOR - c-Met SIGNALING PATHWAY: MOLECULES INVOLVED IN TUMOR METASTASIS

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Signals transduced by Met tyrosine kinase, which is the receptor for scatter factor/hepatocyte growth factor (SF/HGF), are of major importance for the regulation of epithelial cell motility, morphogenesis, and proliferation. In order to define the signalling molecules involved we recently screened for novel substrates of the Met receptor tyrosine kinase (RTK) in the yeast Two-Hybrid-System. We identified a variety of Met receptor interaction proteins (MIPs) that associate with Met in a phosphorylation-dependent manner, e.g. GRP-2, p85 PI3-kinase, SHC, c-srd, and the novel interaction proteins MIP-1, MIP-6 and MIP-7. MIP-1 is the major Met receptor substrate and contains a new class of phosphorylation interaction domain that binds to Met but not to other RTKs. Expression of a dominant interfering MIP-1 mutant abrogated Met-specific downstream signalling. These data suggest a central role of MIP-1 in transmitting Met-mediated cell motility, proliferation and morphogenesis.
Loss of control in Met receptor signaling might also contribute to tumor development and metastasis. Overexpression of SF/HGF is associated with an increased invasiveness and poor prognosis. Experimental evidence further suggests an important role of SF/HGF in tumor growth and metastasis: Transfection of SF/HGF-cDNA into non-metastatic human breast carcinoma cells (MDAMB 435) followed by injection of the cells into mammary fat pad of nude mice resulted in an increased growth rate of the primary tumors which extensively metastasized into lymph nodes and lung.

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FUNCTIONAL PROPERTIES OF CYTOKINES AND THEIR RECEPTORS IN HEPATOBLASTOMA

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The properties of different cytokines for the biological behavior of hepatoblastoma (HB) were investigated. HGF was elevated in serum from 8/13 children with an HB and in 14 patients after tumour resection but not in supernatants of HB cell cultures and serum of xenotransplanted nude mice. With immunoenzymatic staining on 20 HB HGF was detected in the tumours' stromal cells and the HGF-receptor (c-Met) on epithelial HB cells. Thus HGF is produced in HB by a paracrine mechanism and secreted after tumour resection. Proliferation assays will determine the effect of HGF on epithelial HB cells.

Furthermore, our recent studies of 15 HB using immunoenzymatic labelling and analysis of tumour cell lysates as well as culture supernatants indicate that epithelial HB cells are able to secrete IL-1ß, EPO, SCF and TPO and stimulate stromal cells to an enhanced secretion of IL-6, G-CSF, GM-CSF, LIF, M-CSF and SCF. Of these, only IL-6 could be measured elevated in the patients' serum corresponding to thrombocytosis and fever. Hematopietic cells in HB tissue were identified as erythroblasts and megakaryocytes but not granulocyte/monocyte precursor cells. Our results indicate that multipotential hematopoietic stem cells are attracted from the patients' blood stream in HB tissue by virtue of locally secreted hematopoietic cytokines. Here, they proliferate and differentiate to formation of extramedullary hematopoietic foci thereby imitating the

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DNA NUCLEAR CONTENT IN HEPATOBLASTOMA

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Hepatoblastoma is the most frequent malignant liver tumour in infancy and both its biological features and its prognostic behavior are still debated. In particular, no correlation has been noted between DNA nuclear content of the neoplasm, as assessed by flow cytometry, and ist histological type, stage at presentation, and prognosis. In 28 cases of hepatoblastoma, DNA nuclear content and the percentage of cells in the S-phase were assessed by flow cytometry, using formalin-fixed paraffin-embedded archival samples. The PCNA labeling index was also evaluated by immunohistochemistry in the same series of samples, and both flow cytometry and immunohistochemical findings correlated with tumor pathology. The PCNA labeling index was significantly associated with two main histological components (fetal and embryonal) of the tumour. A significant correlation was also found between histological type, DNA nuclear content and the percentage of cells in the S-phase, with aneuploidy and the highest S-phase values proving significantly associated with embryonal cancers.

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Cytogenetic results on hepatoblastoma from the SIOP Tissue bank

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We karyotyped 15 consecutive hepatoblastomas (HB) derived from the European Centers partecipating to the International Liver Tumour Tissue Bank project attached to the SIOPEL 1 clinical trial. The tissue was sent in RPMI 1640 medium; short term coltures were set up from cells obtained by mechanical and enzymatic (collagenase) dissociation. Routine methods for cytogenetic analysis were used. Successful karyotyping was possible in 12 of the 15 tumours. Abnormal karyotypes were detected in 6 cases (see table). 3 cases showed abnormalities of chromosome 2, a trisomy 20. Besides these, the most common abnormality observed was the rearrangment of chromosome 1, as chromosome 1 deletion, translocations and 1q duplication. No recurrent tranlocations, neither double minutes were found.
These data, derived from a large series of HB karyotypes, seem to indicate that karyotyping of HB is possible in the majority of the cases and that abnormalities of chromosomes 2, 20 and possible of chromosome 1 also, are consisting findings in this rare childhood tumour.

pts	age (months)	Histology	Karyotype
1	44	Epithelial Embryonal	50,XY,+2,+6,12q,+20, 22q+,+der(1)t(1;?)(p32;?)
2	18	Embryonal Fetal	53,XY,+7,+8,+8,+19,+20,+21,+der(2)t(1;2)(q21;q36)
3	31	Embryonal Fetal	55,XX,t(1;4)(q21;q32),+2,-4, +1(6p),+7,+8,+15,+17,+17,+19,+20
4	24	Macrotrabecular	46,XY(80%)/50,XY,+8,+16,+20,+22(20%)
5	7	Fetal Embryonal	46,XY,i(1q)(30%)/47,XY,i(1q),+20(70%)
6	23	Fetal	45,XX,-12,-15,+der(12)t(12;15)(q24;q13) fragile site 1q12

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CYTOGENETIC AND MOLECULAR CHARACTERIZATION OF HEPATOBLASTOMA

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Previous reports of cytogenetic abnormalities in hepatoblastoma have consisted of isolated case reports or small series of tumors. The most common recurring cytogenetic abnormalities in hepatoblastoma previously reported are trisomies of chromosomes #2 and #20. The only reported recurring structural chromosome abnormalities, dup(2q) and i(8)(q10), involve only a single chromosome.

In conjunction with centers affiliated with the Pediatric Oncology Group, we analyzed a total of 27 hepatoblastomas using conventional cytogenetic techniques. Fourteen of 27 tumors (51%) demonstrated trisomy of chromosome 2, and/or 20. Nine of the 14 tumors with trisomy 2 and/or 20 also demonstrated trisomy of chromosome 8 and five of these tumors demonstrated trisomies of other chromosomes.

Four cases were characterized by a derivative of chromosomes #1 and #4, an aberration reported only rarely in isolated cases of other types of neoplasms. The abnormality in three hepatoblastomas was der(4)t(1;4)(q12;q34), whereas the fourth case appeared to have breakpoints (q25;q32). All had hyperdiploid tumor karyotypes; however, in the case with (q25;q32) breakpoints, the der(4) was the only abnormality in the stemline. We speculate that the oncogenetic event in our cases may be the loss of a gene or genes on distal 4q or their alteration by juxtaposition to 1q12 heterochromatin. Seventeen other hepatoblastomas retained heterozygosity for microsatellite repeat markers distal to 4q25, supporting the latter possibility.

Further studies will be needed to clarify the clinical significance of these cytogenetic abnormalities.

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Analysis of allelic losses in sporadic and familial hepatoblastoma.

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Although hepatoblastoma (HB) is the most frequent primary malignant liver tumor in childhood, little is known about its molecular pathogenesis. Previous cytogenetic and molecular genetic studies have demonstrated structural alterations of the chromosome arms 1p, 2q and 11p in some HBs. We performed a detailed molecular genetic study of these regions and examined HB samples from 32 patients including 3 familial HBs for loss of genetic material as indicator for the presence of tumor suppressor genes. Loss of heterozygosity (LOH) was studied by PCR-mediated analysis of 32 microsatellite and VNTR sequences from tumor derived and constitutional DNA. In addition, we examined candidate genes in these regions for structural rearrangements, mutations and mRNA expression.

We found no LOH on chromosome 2q or rearrangements of *LCA* or *PAX3*. In contrast, 22% of the cases exhibited LOH on the short arm of chromosome 1 with an overlapping region at 1p36.3. Allelic loss on chromosome arm 11p was detectable in 33% of the samples, the region of overlap confining to 11p15.5. Additional studies on the parental origin of LOH showed that the lost alleles in this region were of maternal origin. mRNA expression of the imprinted *H19* gene was found decreased or not detectable in most cases with LOH of chromosome 11p15.5.

These results suggest that tumor suppressor genes are located in the chromosomal regions 1p36.3 and 11p15.5 and may contribute to the pathogenesis of HB.

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GENOMIC IMPRINTING OF *IGF2* AND *H19* IN HEPATOBLASTOMA

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The human gene for insulin-like growth factor 2 (*IGF2*) is transcribed from four promoters, resulting in at least 6 different mRNA species. The transcription is subject to promoter specific tissue and developmental regulation, as well as to genomic (parental) imprinting. In the normal childhood liver, the *IGF2* gene is transcribed from all four promoters, the P2 - P4 being monoallelically, from the paternal allele, and the P1 being biallelically expressed. The *H19* gene which has been suggested to act as a tumor supressor gene, is located 200 kb downstream of *IGF2*, is monoallelically expressed from the maternal allele, and is in most normal tissues co-regulated with *IGF2* but from the opposite allele. The overexpression of *IGF2* has been implicated in various childhood tumors including Wilms' tumor, rhabdomyosarcoma, neuroblastoma and hepatoblastoma. In Wilms' tumor and rhabdomyosarcoma, frequent loss of *IGF2* imprinting (LOI) leading to biallelic expression of the P2 - P4 promoters has been described, as well as loss of heterozygosity (LOH) involving a duplication of the paternal allele, suggesting a supressor function for a gene(s) on the maternal chromosome. In neuroblastoma however, the *IGF2* over-expression has not been attributed to LOI. We have investigated the expression pattern of *IGF2* at the promoter level and of *H19*, as well as the imprinting status in some hepatoblastoma tumors from patients 9 months to 3 years old. In all cases there was a 2.5 - 5 fold upregulation of total *IGF2* transcription, a downregulation of promoter P1 and activation of P3 compared to the normal counterpart liver tissues. In one of three patients a loss of imprinting of *IGF2* was found. This LOI was related to all three active promoters, in contrast to the situation in the normal liver where P1 alone was biallelic. *H19* expression levels were greatly downregulated in all tumors but a very low monoallelic expression detected by RT-PCR was present. Unlike the situation in Wilms' tumor, no differences in methylation pattern between the normal and tumor tissues were observed in the *H19* promoter or 3' region in any of the tumors. We suggest that *H19* expression is not necessary for maintaining a monoallelic *IGF2* expression. The methylation pattern in the *IGF2* P3 region will be discussed.

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HEALTH-RELATED QUALITY OF LIFE IN CHILDREN WITH CANCER

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The prospects for long-term survival continue to improve for children afflicted by cancer, reflecting ever-more successful strategies of treatment aimed at eradication of malignant disease. As a result, increasing attention is being paid to the late effects of therapy and to the burden of morbidity associated with active therapeutic intervention. These adverse consequences compromise health status and reduce health-related quality of life (HRQL).

Health status can be viewed as an amalgam of components, each of which may be affected adversely and to different degrees by cancer and its treatment. These components (known also as dimensions, domains or attributes of health) include sensation (vision, hearing, speech), mobility/ambulation, emotion, cognition, dexterity and pain/discomfort. Each of these attributes can be described by a series of categorical levels, varying from normal to severely limited. In this multi-attribute approach to health status measurement, the combination of one level for each attribute represents a composite health state.

Measures of health status and HRQL must have the properties of reliability, validity and responsiveness. Few such instruments have been devised for use with children. In the pediatric context, particular challenges are posed, including - identification of the relevant attributes, accommodation for developmental age, implementation of self-report for health status, and the estimation of appropriate cardinal preference (utility scores) for health states. Information about the health status of patients can be collected from one or more viewpoints: those of patients, patients and a variety of health professionals. Each of these viewpoints is legitimate, and important insights have been obtained by comparing results from the various perspectives.

A multi-attribute preference-based system for assessing HRQL has been

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applied to survivors of standard and high-risk acute lymphoblastic leukemia (ALL), Wilms' tumor and neuroblastoma, and to children who have completed treatment for brain tumors. As anticipated, the last group exhibits a considerable burden of morbidity. In addition, this system has been used to measure HRQL in children with ALL on active therapy. It is important to interpret the results of such studies in light of the HRQL experienced by comparable members of the general public, as has been determined in population surveys.

The results of these studies will be presented and discussed in detail. Among the main findings are the responsiveness of the instruments (Health Utilities Indexes Mark 2 and Mark 3 - HUI2 and HUI3) to changes in health status during active therapy; the paucity of morbidity in survivors of standard risk acute lymphoblastic leukemia; and the prevalence of pain in children who were treated for brain tumors.

The multi-attribute approach to health status measurement provides a standard framework for describing the number and severity of multiple morbidities in individuals. Measures of health status can be used for discriminative, evaluative or predictive purposes. The first affords the ability to detect differences within and among populations; the second focuses on the assessment of changes in health status within an individual over time; and the third addresses prognostic considerations. Measurement of HRQL is useful not only in clinical management but also in pharmaco-economic evaluation, assessment of quality of care and the development of health care policy.

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MECHANISMS INVOLVED IN THE METASTASIS OF CANCER TO BONE

F. William Orr, O. Sanchez-Sweatman, P. Kostenuik, and G. Singh. University of Manitoba and McMaster University, Winnipeg and Hamilton, Canada

Continued

Clinically, osteolysis dominates the presentation of bone metastases. Tumor cell-derived cytokines can mediate bone resorption through osteoclast activation and macrophage recruitment. Tumor-derived matrix metalloproteinases can act directly on bone matrix to cause its degradation. These destructive processes release bone-derived growth factors and matrix constituents which, in turn, promote expression of the metastatic phenotype. In some tumors, local osteoblastic proliferation can be attributed to production of urokinase plasminogen activator. Although none of these mechanisms is responsible alone for the development of bone metastases, their elucidation has suggested novel therapeutic approaches such as inhibitors of bone resorption, angiogenesis, or proteases that might be used to block specific steps in a prophylactic manner.

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MECHANISMS INVOLVED IN THE METASTASIS OF CANCER TO BONE

F. William Orr, O. Sanchez-Sweatman, P. Kostenuik, and G. Singh. University of Manitoba and McMaster University, Winnipeg and Hamilton, Canada

Bone metastases are common and cause morbidity in children with neuroblastomas, Ewing's tumor, and the bone metastasizing renal tumor of childhood. In adults, carcinomas of the breast, prostate, lung, kidney and thyroid typically have a marked osteotropic propensity. Recent clinical and experimental observations on bone metastasis have provided important information on its pathogenesis. Subclinicalhematogenous seeding of bone marrow by micrometastatic cancer cells can be detected by molecular or immunocytochemical methods at the time of primary tumor diagnosis in circa 1/3 of patients. In reality, the clinical presentation of bone metastases with pain, osteolysis, or fractures represents a very late stage phenomenon resulting from subsequent synergistic cellular and molecular interactions between such cells and the unique bone microenvironment. The preferential localization of cancer cells in bone may be directed by bone-derived chemoattractants which include defined matrix constituents and growth factors. Their subsequent attachment to type I collagen in the marrow stroma or bone matrix are mediated by specific adhesion molecules such as the $\alpha 2\beta 1$ integrin, the expression of which is upregulated by matrix itself or by TGF-β1, a major bone-derived growth factor. Integrin-mediated cell attachment is likely to stimulate cell growth through cellular signal transduction pathways. Cells located in proximity to the bone trabeculae experience a growth advantage which can be related to release of growth factors from the bone matrix.

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RAS ONCOGENES AS AGENTS OF ANGIOGENESIS AND MULTICELLULAR SURVIVAL: IMPACT ON THE DEVELOPMENT AND SPREAD OF SOLID TUMORS

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Background: The vast majority of mutant oncogenes or over-expressed proto-oncogenes are thought to contribute to the development of malignant tumors by *directly* promoting uncontrolled ("rampant") cell growth. *Ras* oncogenes - which are detected in about 30% of all human cancers - are a case in point. Normal Ras proteins are thought to function primarily as relay switches to convey growth promoting signals from upstream activated cell surface growth factor receptor protein tyrosine kinases to downstream cytoplasmic serine threonine kinases and then nuclear transcription factors. The latter, when activated, can trigger the expression of various target genes which can promote cell growth, such as those encoding autocrine growth factors. Unlike normal Ras proteins, mutant forms can function in a constitutive, activated manner, to promote growth, even in the absence of growth factors binding to their respective tyrosine kinase receptors. There is now considerable worldwide interest in targeting proto-oncogenes (eg. the EGF and neu/Her-2 receptor tyrosine kinases) or mutant oncogenes such as *ras* as a new means of cancer therapy. A number of promising agents have been developed, eg. ras farnesyltransferase (FTIs), and neutralizing antibodies to the EGF receptor or the neu/Her-2 proto-oncogene. These agents appear to have in common the property of being modestly cytostatic; they are not cytotoxic for target tumor cells grown in monolayer cell culture. Nevertheless, they can sometimes match the anti-tumor effects of conventional cytotoxic drugs against large established solid tumors and even cause massive regressions in preclinical models. This surprising result suggests that such drugs function not only as growth inhibitors but in other ways so as to acquire cytotoxic properties in vivo.

Hypothesis: Proto-oncogenes and mutant *ras* oncogenes can promote the growth of solid tumors in vivo by inducing or facilitating angiogenesis

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RAS ONCOGENES AS AGENTS OF ANGIOGENESIS AND MULTICELLULAR SURVIVAL: IMPACT ON THE DEVELOPMENT AND SPREAD OF SOLID TUMORS

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and inhibiting apoptosis, in addition to promoting cell growth. Therefore drugs which target oncogenes or proto-oncogenes may function in vivo as anti-angiogenic and pro-apoptotic agents.

Results: Using *ras* gene transfection and "knockout" strategies in both rodent and human carcinomas tumors, evidence has been obtained to show that *ras* oncogenes induce or upregulate vascular endothelial growth factor (VEGF). VEGF is currently thought to be *the* major tumor angiogenesis growth factor in most types of human cancer, and has no known autocrine function since tumor cells do not express VEGF receptors. Activated endothelial cells express the greatest levels of such receptors, including those found in new tumor blood vessels. Evidence was also obtained for an "anti-apoptotic" function for mutant *ras*, but this was detected only in the context of multicellular "spheroid" cell cultures - which mimic the three dimensional nature of avascular microscopic metastases. On the basis of these results we reasoned that at least part of the therapeutic effect obtained in vivo of drugs such as *ras* inhibitors could be due to inhibition of tumor angiogenesis and induction of apoptosis, both of which would endow such drugs with cytotoxic properties. Evidence for both possibilities, using Ras FTI's, has been obtained, and will be summarized.

Implications: (i) **For experimental therapeutics**: many other different drugs, which target other oncogenes, may function in a similar manner in vivo to inhibit tumor growth; (ii) **For metastasis**: oncogenes may promote hematogenous metastasis by helping to make it possible for tumor cells to form and survive as multicellular microemboli - which are known to be much more efficient at forming distant metastases. Oncogenes may also facilitate the subsequent growth of such nascent metastatic tumors by promoting angiogenesis.

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Anti-angiogenesis: a novel therapeutic concept in pediatric oncology ?

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Angiogenesis, the formation of new capillaries from existing vessels, occurs by the invasion and migration of capillary endothelial cells into adjacent tissues with subsequent endothelial cell proliferation. Normal angiogenesis is prominent during embryogenesis but essentially ceases in adulthood. Pathological angiogenesis is particularly prominent in solid malignancies of childhood where it probably contributes to tumor growth and hematogenous metastasis. There is growing evidence that tumor angiogenesis may have pathogenetic, diagnostic, prognostic and therapeutic implications for solid pediatric malignancies:

Pathogenesis: Growth of pediatric tumor cells and metastasis of the resulting tumors can be suppressed by specific inhibitors of tumor-derived angiogenesis stimulators.

Diagnosis: Suborbital hematomas or bloody urine suggest the presence of vascularized tumors, i.e., advanced neuroblastoma or nephroblastoma, respectively.

Prognosis: The number of capillaries and the levels of angiogenesis factors in pediatric tumors correlate with poor prognosis. Highly vascular tumors like teleangiectatic osteosarcoma have a poor prognosis.

Therapy: In experimental systems, specific angiogenesis inhibitors alone can inhibit tumor growth and potentiate the effects of chemotherapy, radiotherapy or hyperthermia.

Work of our laboratory has focussed on the identification of angiogenesis inhibitors. We have identified several molecules so far, some of which may be down-regulated by the over-expression of an oncogene. It is possible that pediatric malignancies achieve their neovascularization by down-regulating endogenous angiogenesis inhibitors. The therapeutic application of angiogenesis inhibitors may be of benefit in pediatric oncology.

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NOVEL STRATEGIES IN RADIATION ONCOLOGY

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During the last two decades significant changes have occured in radiation oncology parallel to the improvement of treatment results within multidisciplinary treatment strategies in pediatric oncology which have often involved radiotherapy.

These changes referred to better delineation of target and critical organs, first by integration of CT into fluoroscopic simulation based treatment planning in the late 70ies and 80ies. Recently, MRI has been introduced. Functional imaging like PET will be explored in future. 3 D-treatment planning is about to be put into general clinical practice, which means 3 D-assessment of anatomy - including target and critical organs - as well as 3 D-display of radiation dose distribution. Based on dose volume histograms tumor control (TCP) and normal tissue complication probabilities (NTCP) can be calculated using algorithms derived from clinical experience.

3 D-treatment planning based conformation radiotherapy (CR) is being introduced for various tumor sites at present. CR means a strong correlation between target volume and volume treated by irradiation, through individual blocking in multiple fields or multileaf collimation of the radiation beams. In parallel, sophisticated qualitiy assurance procedures are being explored, which are mainly "on line portal imaging" and "in vivo dosimetry".

Stereotactic (fractionated) radiotherapy for brain tumors represents one most precise variation on CR, which is going to be extended to sarcoma of the head, and may be applied for extracranial tumors in future, too.

Similiar developments have taken place in treatment planning and delivery of intracavitary and interstital brachytherapy, leading to new approaches, in particular in pediatric sarcoma treatment.

Some further experience will be interesting from the application of proton and heavy ion beams, which are advantageous to conventional photon radiotherapy because of their physical and biological properties, i.e. better dose distribution and higher biological effectiveness.

Unconventional fractionation schedules (e.g. accelerated hyperfractionation) have been introduced into clinical practice, but will need further clinical and biological evaluation, e.g. by correlating in vivo measurements of proliferative activity to clinical outcome.

Individualization of treatment based on radiobiologically relevant prognostic factors - on the cellular and molecular level - will have a major impact on radiotherapy in the next decade:
among these are individual measurements of tumor radiosensitivity, tumor proliferation rate and hypoxia as well as of normal tissue radiosensitivity.

Modification of tumour and normal tissue response to irradiation will probably become a tool in future by altering the level of radiation induced DNA-damage, by changing the pattern of DNA repair, and by influencing molecular pathways of radiation through cytokine interaction, gene induction, and genetic manipulations.

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GENOMIC IMPRINTING: BASIC CONCEPTS AND ITS ROLE IN THE PATHOGENESIS OF NEOPLASTIC DISEASES

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The concept of genomic imprinting is based on the perplexing observation that some parts of the genome may behave differently depending on whether they are of paternal or maternal origin. From a mechanistic point of view, it is a process that leads to the transcriptional silencing of one of the allels in a subset of genes which is regulated in a parent of origin specific manner and which results in functional hemizygosity. This paternal/maternal disparity explains why mutations may have different effects on the paternally and maternally derived parts of the genome. Consequently, these differences have many important implications for chromosomal diseases, for numerous inherited disorders associated with physical and mental impairments, the etiology of developmental defects, but also for cancer predisposition syndromes and tumorigenesis in general. As a result, genomic imprinting has necessitated the refinement of the laws of Mendelian inheritance and the supplementation of the traditional models of unusual inheritance patterns in familial cancer predisposition syndromes, such as the Knudson's "two hit" hypothesis.

The imprinting process consists of an epigenetic component, that marks one parental chromosome and a genetic component (DNA) that is modified by the imprint. The best candidate with all the required properties is DNA methylation. This notion is supported by the fact that all imprinted genes examined so far contain DNA sequences which are methylated in a parental-specific manner. In addition to their unequal methylation and parent-specific monoallelic expression, imprinted genes also

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exhibit an unusual asynchronous replication behaviour. These three features contribute to the control and flexible regulation of transcription. However, it is not yet clear whether these three phenomena are related to one another by a common mechanism.
It has been estimated that there are only approximately 100 imprinted loci throughout the whole genome. To date, 16 imprinted genes have been described in mice and humans, 5 of which are maternally expressed and 11 are paternally expressed. They provide developmentally important functions for fetal growth, viability and behaviour after birth. In particular, placental development appears to depend on paternally expressed genes, whereas embryonic growth on maternally expressed genes.
Disruption of the normal imprinting signal by homozygous inactivation, relaxation of imprinting or altered interactions between imprinted genes perturb normal development and make the affected tissue more susceptible to mutagenic environmental factors. Such epigenetic modifications can therefore be considered as an initiating event in tumorigenesis. In the context of tumor development, the effects of genomic imprinting become especially evident in two different types of tumors that are exclusively comprised of genetic material from one parent: the hydatidiform mole (paternal components) and the ovarian teratoma (maternal components). However, there also is accumulating evidence that defective imprinting plays an essential role in many other neoplasms, particularly those encountered in very young children, such as Wilms' tumor, neuroblastoma rhabdomyosarcoma and hepatoblastoma. Since methylation defects may represent the earliest and most ubiquitous phenomena in tumor development, it can be expected that epigenetic alterations of imprinted genes also trigger initiation and progression of a variety of other neoplasms.

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Genomic imprinting and DNA methylation in neuroblastoma

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Our data suggest that chromosome 1p harbours 2 different suppressor loci inactivated in N-myc amplified and N-myc single copy neuroblastoma. In the N-myc single copy tumours the commonly deleted region maps to 1p36.2-3, whereas the deletions in N-myc amplified neuroblastomas extend from the telomere to at least 1p35-36.1. Our studies on genomic imprinting further support this view. Genomic imprinting is the phenomenon whereby two alleles of a gene are differentially expressed depending on their parental origin. Preferential loss of one parental allele of a tumour suppressor region is considered as an indication of genomic imprinting of the involved suppressor gene. We found that the lost 1p alleles were of preferential maternal origin in the N-myc single copy cases (16/17 cases) and random origin in the N-myc amplified cases (18/30 maternal LOH). This suggest that the distal 1p suppressor inactivated in N-myc single copy neuroblastoma is imprinted.

Furthermore, we mapped a modifier of methylation (MEMO-1) to 1p35-36.1. In neuroblastoma cell lines we found methylated HLA I genes to be expressed and hypomethylated alleles to be silent. All cell lines with the hypomethylated phenotype have large 1p deletions. Cell lines without 1p deletions or with distal deletions have the normal methylated phenotype. In the mouse a modifier of methylation (SSM-1) has been genetically mapped to a chromosomal region syntenic to human 1p36. This strongly suggests that a gene that modifies the methylation status of HLA class I genes maps in 1p35-36.1. Genomic imprinting is thought to be mediated by differential methylation of the parental alleles of a gene. Therefore, MEMO-1 could be functionally related to the imprinted distal 1p neuroblastoma suppressor locus and/or to the N-myc gene, of which preferential amplification of the paternal allele has been reported.

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THE IMPRINTED *IGF2* AND *H19* GENES IN CANCER.

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The phenomenon of genomic (parental) imprinting was quite recently recognized as a violation of Mendel's laws, resulting in the unequal contribution of parental autosomal gene expression. Imprinted genes are thus normally expressed from only one of the parental chromosomes and it is always the same parental allele that is expressed for a given imprinted gene. The genes for insulin-like growth factor 2 (*IGF2*) and its relatively close neighbour *H19*, are imprinted so that they are expressed from the paternal and maternal chromosomes respectively. Both these genes have been implicated in various forms of tumors, and their expression levels have been studied in a number of different forms of cancer, both childhood and adult. The *IGF2* gene product has been shown to act as a mitogen and survival factor. It is frequently overexpressed in many tumors and the monoallelic expression is sometimes lost (LOI). Tumors showing overexpression with LOI include rhabdomyosarcoma, Wilms' tumor, hepatoblastoma, Ewing's sarcoma, leiomyosarcoma, cervical carcinoma, lung cancer and in rare cases of hepatocellular carcinoma. Overexpression without LOI has been determined in neuroblastoma and leiomyoma. In addition there are a number of tumors showing *IGF2* overexpression where LOI has not been determined i.e. pheochromocytoma, colon carcinoma, breast cancer and liposarcoma.
The *H19* gene, located 200 kb downstream of *IGF2*, has been suggested to function as a tumor suppressor gene at the RNA level, since the mRNA is not translated. It seems to have priority over *IGF2* on at least one shared enhancer element which is used by the *IGF2* promoters P2 - P4. The *H19* promoter is normally silenced by methylation on the paternal allele allowing *IGF2* to be transcribed. Overexpression and LOI of the *H19* gene has also been shown for a number of tumors. Among these are lung cancer, choriocarcinoma, esophageal cancer, cervical carcinoma and some Wilms' tumors, which show both overexpression and LOI. In hepatoblastoma however, an almost total downregulation of *H19* coupled with retention of imprinting was demonstrated. It seems to be a contradiction in terms, that overexpression of a tumor suppressor gene is present in tumors. It is possible however, that the tumor suppressor activity is tissue-specific or that other factors may influence its action so that an overexpression actually gives a growth advantage. Another possibility is that a mutation in the *H19* gene renders it inactive as a suppressor gene but may give it other charachteristics which effect the overall growth advantage.
Since cytosine methylation is involved in both silencing and activation of imprinted genes, it is likely that the adverse methylation seen in tumor cell DNA may affect imprinting as well as the expression level of these genes. We have investigated the methylation patterns of *H19* and *IGF2* in hepatoblastoma and hepatocellular carcinoma. These results will be discussed in relation to the normal methylation patterns during human liver development.

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MOLECULAR GENETIC AND BIOCHEMICAL ALTERATIONS IN CHILDHOOD PRELEUKEMIA

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The childhood preleukemias include juvenile chronic myelogenous leukemia (JCML), bone marrow monosomy 7 syndrome (Mo 7), and adult-types of CML and MDS. JCML and Mo 7 account for 80% of cases of preleukemia in children < 5 years old. Childhood preleukemia is associated with certain genetic predispositions including neurofibromatosis, type 1 (NF1), severe congenital neutropenia (SCN), familial Mo 7, Fanconi anemia, and Bloom syndrome. Our laboratory has been working to characterize the genetic and biochemical basis of preleukemia in children for the past few years. The NF1 gene *(NF1)* encodes a protein (neurofibromin) that acts as a GTPase activating protein (GAP) for Ras family proteins. Ras proteins transduce signals in myeloid cells by cycling between an inactive, GDP-bound form and an active, GTP-bound conformation that promotes growth. The GAP activity of neurofibromin suggested that *NF1* might function as a tumor-suppressor gene in myeloid cells by down-regulating Ras. Our genetic and biochemical data support this model as we have shown: (1) loss of the normal *NF1* allele in the bone marrows of approximately half of the leukemias that arise is children with NF1; (2) homozygous inactivation of both *NF1* alleles is a subset of cases; (3) activating *RAS* mutations in approximately 25% of children with JCML or

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Mo 7 but not in NF1 patients with these disorders; and, (4) decreased NF1-associated GAP activity, elevated levels of Ras-GTP, and MAP kinase activation in primary bone marrow cells from children with NF1 and myeloid disorders. Mice that are heterozygous for a targeted mutation at murine *NF1* are susceptible to myeloid leukemia. Fetal liver cells from embryos with homozygous inactivation of murine *NF1* show an aberrant pattern of CFU-GM colony formation in response to GM-CSF that is remarkably similar to that seen in JCML. Transplantation of these fetal liver cells into irradiated recipients induces a myeloproliferative disorder that is reminiscent of JCML. We have also detected activating *RAS* mutations and chromosome 7 deletions in a subset of children with SCN who developed preleukemia or myeloid leukemia while receiving G-CSF. Taken together, these data: (1) implicate *NF1* as a tumor-suppressor in immature myeloid cells which functions by negatively regulating Ras; and, (2) suggest that hyperactive Ras plays a central role in the development of childhood preleukemia. We are using our NF1 mouse model to test novel anti-Ras therapeutics.

I wish to acknowledge the support of pediatric oncologists throughout the world for providing specimens for our studies; my collaborators and the members of my laboratory.

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Pathways to Obtaining Clinical Benefits from Cancer Susceptibility Genes

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We have recently set up a drug discovery program to explore new methods for identifying targets for anti-cancer agents. Working from the assumption that tumor cells are genetically unstable, we have focused on methods to take advantage of the accompanying defects that exist in damage response pathways. Because these damage response pathways have been carefully studied in organisms such as S. cerevesiae, we have taken advantage of this pre-existing set of knowledge. Several examples will be given of how this information can be used to validate both targets and compounds. The implications regarding molecular diagnostics will be stressed. We see these approaches as tackling some of the need to identify agents specific for molecular defects, and in providing early target validation.

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DIAGNOSIS OF BONE TUMOURS

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Primary bone tumours are comparatively rare and accurate diagnosis is paramount in the management of patient's with such tumours. It is important that the diagnosis of a bone lesion is made within a team framework and should not be left to the pathologist or radiologist individually. It is essential that the pathologist works in close co-operation with the radiologist, surgeon and oncologist. In many cases the diagnosis can readily be established on biopsy material but there remain cases which prove to be a challenge even to the most experienced pathologist. The introduction of newer imaging techniques such as magnetic resonance imaging and advances in immuno-histochemistry have improved the diagnosis and understanding of primary bone tumours. Advances in cytogenetic chromosomal investigations has added understanding to the categorisation of some tumours and has enhanced diagnosis. Fluorescent in-situ hybridisation has enabled pathologists to demonstrate chromosomal translocations on tiny samples and has obviated the need for tumour culture in order to demonstrate these. Such techniques have proved invaluable, particularly in the differential diagnosis of the round cell tumour group.

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DIAGNOSIS OF BONE TUMOURS (2)

Dr Archie J Malcolm

Pathologists are increasingly contributing to assessment of prognostic factors in bone tumours. It is already well established that the percentage necrosis of osteosarcoma following chemotherapy is a significant prognostic factor. Similar findings have been established in other tumours, particularly malignant fibrous histiocytoma and Ewing's tumour. Ideally it would be useful, if at the time of biopsy, an accurate prediction of chemosensitivity could be established. There is some evidence that p-glycoprotein expression may be associated with a higher relapse rate and a worse outcome. Similarly there is some suggestion that the higher the mitotic:apoptotic ratio the worse the response to chemotherapy. Further molecular developments will allow prediction of chemosensitivity of tumours and provide a more scientific basis for diagnosis and staging.

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Tumour Resection and Prosthesis in the Therapy of the Osteosarcoma

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With administration of aggressive chemotherapy regiments, early resection after chemotherapeutic pretreatment is routinely performed for osteosarcoma and Ewing's sarcoma in special therapy centers, and different forms of reconstruction are available.
Apart from custom-made and modular tumour endoprostheses with or without extendable modules, there are also alternative methods that are still used in resection treatment. In the knee region, these are above all the so-called rotation-plasty and the resection arthrodesis; also, in the shoulder region, a resection arthrodesis or homologous bone and joint transplants, and autologous transplants, mainly from the fibula, with and without vascular reconstruction for the graft. In big tumours the resection-replantation with shortening of the arm is possible. In the pelvic region, if not a hemipelvectomy is mandatory, resections were chiefly performed without reconstruction. Experience in 131 cases of osteosarcoma and 114 cases of Ewing's sarcoma under the age of 21 are reported.

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MOLECULAR BASIS OF EWING'S TUMOR

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The Ewing family of tumors (ET) belongs to the heterogeneous group of small round cell tumors in which the phenotypic distinction between the various entities of this spectrum can be difficult. Identification of a recurrent cytogenetic abnormality, a balanced t(11;22)(q24;q12) in ET has provided with a genotypic marker. Characterization of this translocation revealed that it results in the fusion of the EWS gene, encoding a new RNA binding protein, localized on chromosome 22 with the FLI-1 gene, encoding a transcription factor of the ETS family, on chromosome 11. Further studies have shown that, in some cases, the EWS gene may be fused with other members of the ETS family including ERG on chromosome 21, ETV1 on chromosome 17 and E1AF on chromosome 7. These gene fusions can be detected with the specific and sensitive RT-PCR method.

A total of 326 tumors for which the diagnosis of ET was suspected on a clinical and/or radiological basis have been analysed with RT-PCR in search of the various fusion transcripts of EWS with ETS partners (EWS FT). 19 cases (6%) were not evaluable due to either degradated RNAs or absence of tumor cells in the studied sample.

- 189/207 (92%) tumors diagnosed as Ewing's were positive for the EWS FT.
- none of the 63 tumors with a diagnostic clearly different from that of Ewing's tumor was positive for EWS FT (osteosarcoma, 15; benign pathologies, 14; lymphomas, 7; central PNET, 6; other diagnosis, 21).
- tumors which belonged to the differential diagnosis of ET were occasionally positive: 1/7 rhabdomyosarcoma, 1/4 neuroendocrine carcinoma, 7/20 undifferentiated sarcoma, 2/6 MIBG-, catechol neuroblastoma.

These results indicated a good agreement between phenotypic and genotypic diagnostic criteria in ET. However, some discrepancies were observed. Absence of EWS FT in some cases diagnosed as ET might be related either to a pathological diagnosis by exces or to a default in the genotyping analysis. In this respect, further analysis of the EWS FT negative ET revealed new molecular variants. In one case, the breakpoint within the EWS gene was located proximally to the EWSR1 breakpoint cluster region impeding the detection of the EWS FT by previously described primer set. In two other cases, the EWS gene was fused with a new member of the ETS family of transcription factor which was termed FEV for Fifth Ewing Variant. The recognition of such rare variants is important since it leads to a progressive improvement of the reliability of the molecular diagnosis of ET which is becoming more and more used as a routine test. It also shows that the various entities of the Ewing spectrum of tumors are generated through a very homogeneous oncogenic alteration which consists of the fusion of EWS with various but similar ETS DNA binding domains, although this abnormality can arise through different chromosome rearrangements. This abnormality permits accurate distinction of ET from phenotypically overlapping spectrum of tumors. In this series, respective frequencies of the different EWS FT was EWS/FLI-1 type 1, 99 (50%); type 2, 33 (17%); other types, 43 (21%); EWS/ERG, 23 (12%); EWS/ETV1, 2 (<1%); EWS/FEV, 2 (<1%). No fusion of EWS with E1AF was observed. No evident association between the particular EWS FT and localization, phenotypic trait or evolution of the disease was observed.

Since fusion transcripts provide with a sensitive marker for tumor cells, we have developped an assay to search for the presence of Ewing's cells in blood, bone marrow and stem cell samples from patients with Ewing's tumor. Preliminary results from this study will be presented.

Interestingly, various chromosome translocations have recently been characterized in solid tumors, suggesting that such diagnostic tests and assays for detection of minimal and residual disease might be used in other malignancies as well.

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CLASSIFICATION AND STAGING IN EWING'S TUMOR

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Ewing's sarcoma and the other members of the small round-cell tumors define a group of pediatric neoplasms that may cause diagnostic difficulty because they are often indistinguishable using light microscopy. These tumors represent approximately 15% of all childhood neoplasms. Although classic diagnostic methods (light and electron microscopy) are highly suggestive of the tumor type, a more precise diagnosis and classification is of utmost importance. This group of small cell tumors exhibit highly characteristic cytogenetic abnormalities increasing the identification of the exact tumor type. Inadequate amount and the poor quality of the fresh tissue available for such karyotyping makes cytogenetic analysis successful in only 50% of Ewing's sarcomas. The recently introduced techniques of fluorescence in situ hybridization (FISH) and the polymerase chain reaction(PCR) improved the molecular genetic characterization of even small amounts lesional tissue samples. This neoplasm is closely related to a varied group of small round-cell tumors collectively designated as "peripheral primitive neuroectodermal tumors" (PNET). These tumors are characterized by a constant recurrent chromosomal translocation, t(11;22) (q24;q12) and the expression of the glycoprotein p30/32 encoded by the MIC2 gene. Such features not only aid diagnosis, but establish a tumor spectrum, forming the Ewing family of neoplasms. Several monoclonal antibodies which identify different epitopes of this antigen have been developed; most of them employed HBA 71 or 013 in the study of the Ewing family of lesions.

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CLASSIFICATION AND STAGING IN EWING'S TUMOR

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More than 90% of these tumors show immunoreactivity with HBA 71, but various non-neoplastic tissues and other unrelated tumors can on occasion give positive results. Among these are alveolar and embryonal rhabdomyosarcoma, and non-Hodgkin's lymphoma. The positive staining pattern, however, in such tumors is different from that observed in Ewing's sarcomas. Studies employing 013 immunostaining showed this antibody to be 100% sensitive for Ewing's sarcomas. No staining was detectable in cases of neuroblastoma, and non-lymphoblastic lymphoma but false positive reactions were seen in lymphoblastic lymphoma and a small number of non-Ewing tumors. The prognostic significance of separating Ewing's sarcoma and PNET is getting clearer. Although poorer prognosis has been associated with neuroectodermal differentiation, however more recent trials using intensive multi-agent chemotherapy protocois strongly suggest that the survival of patients with PNET is comparable to those Ewing's sarcoma patients of a similar clinical stage. The results from the current intergroup studies in the U.S.A. also failed to detect any prognostic significance in grouping these tumors into separate groups of neoplasms. Impressive improvements have been achieved in the diagnosis, treatment and prognosis of children and adolescents with small round-cell tumors resulting in an increased importance on more precise diagnostic procedures such as electron microscopy, immunohistochemistry, cytogenetics and molecular genetics. These, as well as the recognition of novel phenotypic tumor characteristics has had and will have a major impact on the diagnosis of malignant small, round cell tumors of bone and soft tissues

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CHEMOTHERAPY IN EWING TUMOURS.

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The prognosis for patients with Ewing tumours has impressively improved. With the use of combination chemotherapy, disease-free survival rates between 50 and 70 % can be considered standard. Most cooperative groups apply four-drug combination chemotherapy regimens including alkylating agents, anthracyclines, vinca alkaloids, and actinomycin D. Regarding alkylating agents, at present ifosfamide is more widely used than cyclophosphamide. The risk-benefit ratio regarding better results or more toxicity needs to be investigated. The additional etoposide as fifth agent or replacing actinomycin D is also under current investigation, again weighing the benefit of possibly better results against the possible risk of an increased incidence of second malignancies. For local treatment, radiation, surgery, or the combination of both are being used. With longer metastasis-free survival as a result of effective systemic treatment, the risk of local failures following radiation has become evident, advocating an increasing role of surgery. The decision for local therapy is related to initial tumour size, as well as responsiveness to primary chemotherapy; the larger the tumour, the less responsive to primary chemotherapy, the more surgery becomes the treatment of choice to assure safe local control. Whether pretreatment with radiotherapy in addition to upfront chemotherapy allows the more conservative surgical approach, again is under current investigation.
Approximately 20 % of patients present with detectable metastases at diagnosis. The prognosis of these patients is poor, in particular when multiple bone metastases are present. Attempts are currently made to improve the prognosis for these patients with the use of megatherapy regimens followed by blood progenitor cell rescue, in particular in patients where a remission of disseminated disease cannot be reached with conventional treatment modalities. Following relapse, again the prognosis is extremely poor, in particular in patients relapsing within 2 years from initial diagnosis. Less than 10 % of these patients survive longer than 2 years following relapse. Patients responsive to second-line systemic chemotherapy again may benefit from consolidation megatherapy regimens followed by blood progenitor cell rescue.
The diagnosis of Ewing tumours has recently been complemented by the molecular detection of fusion transcripts from the Ewing tumour characteristic chromosomal translocations, most commonly t(11;22). Early detection of blood and/or bone marrow contamination with tumour cells may lead to a new clinical staging system allowing stratification of treatment intensity. Prospective studies, however, are needed to demonstrate that patients with molecular detection of disseminated disease are patients at risk for relapse.
In summary, standard risk Ewing tumour patients may be cured with conventional combination chemotherapy regimens and preferably surgery for local control with or without additional radiation. New approaches are needed for high-risk patients, e.g. patients with synchronous metastases or poor response to conventional chemotherapy.

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RADIOTHERAPY IN EWING'S SARCOMA

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Radiotherapy can be used either alone or in combination with surgery as local treatment in Ewing's sarcoma. On the basis of the CESS-data, combined surgery plus radiotherapy in pelvic tumors yielded survival figures comparable to lesions in favourable sites and this combined approach seems to be useful in poor prognostic lesions (large volume, critical site with unradical resection). Radiotherapy alone is indicated in lesions which cannot be completely resected (e.g. spine, skull) or where surgery would be severely mutilating. The optimal dose is probably in the range of 45 to 55Gy in children and adolescents depending on response to chemotherapy and initial tumor volume. Individually shaped radiation fields with less generous safety margins than usually recommended are probably possible if an extensive intramedullary involvement has been excluded by adequate diagnostic staging including MR-tomography. Side-effects of radiation treatment are comparable to those of surgery. An increased risk of second malignancies after radiotherapy has not been found in the CESS-studies up to now.

In patients with initial lung metastases, additional lung irradiation with 15-18Gy after CR probably increases long-term survival (Dunst et al., Strahlenther.Onkol. 169,621-3,1993). This seems to be as effective as high-dose chemotherapy with BMT, but less toxic. In case of bone metastases, high-dose chemotherapy + BMT/PSCS with additonal local irradiation (or surgery) of all bony lesions, if possible, may cure a subset of patients.

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LANGERHANS CELL HISTIOCYTOSIS

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Langerhans cell histiocytosis (LCH) describes a variety of clinical diseases, historically variously named: eosinophilic granuloma, Abt-Letter-Siwe syndrome, histiocytosis X, etc. The lesion consists of an accumulation of dendritic cells with Langerhans cell phenotype. The etiology is still unknown and may reflect a cytokine-mediated proliferation of Langerhans cells or a reactive process following a viral infection. Recent findings have led to the assumption of a clonal neoplastic disorder. The non-familial and rare disease (annual incidence 0.2 - 1.0 per 100.000 children under 15 years) may occur in all age groups but predominantly affects infants and young children. The clinical presentation ranges from a spontaneously regressing solitary bone lesion to a generalised life-threatening disorder. Intermediate forms are usually characterised by a chronic course. The lesions most frequently affect skeleton, skin, soft tissue, liver, spleen, lymph nodes and lungs. Other rare manifestations may occur in the gastrointestinal tract, thymus, endocrine system and the CNS, the latter usually with an intriguing slowly progressing course. Multi-organ disease is often associated with dysfunction of liver, lungs or haematopoetic system.
Recently efforts have been made to establish generally accepted criteria for diagnosis and staging. For a definite diagnosis light microscopical and immunohistochemical findings and ultrastructural features are essential (either demonstration of CD1a positivity or the presence of Birbeck-granules in LCH cells). According to signs and symptoms at presentation, the disease can be divided into two categories with subsets: 1) single-system disease with single site or multiple site lesions and 2) multi-system disease when more than one organ or organsystem are involved. Patients with single system disease (usually bone, lymph node or skin) generally have a high chance of spontaneous remission and favourable outcome. For these children various therapeutic principles have emerged from clinical experience with intralesional steroid infiltration, surgical excision or low radiation therapy as well as topical skin treatment with steroids, mustargen, or PUVA. The use of chemotherapy including cytostatic agents is generally accepted in the treatment of patients with multi-system disease, but the best drug or regimen for a defined risk group remains controversial. Disseminated LCH in young children (< 2 years) with multiple organ involvement and presence of organ dysfunction can be very difficult to treat. The risk of fatal outcome is high. Ongoing research for new agents in addition to the widely accepted drugs, vinblastine (VBL) and prednisone (PRED), has led to the use of etoposide (VP16), 2-CDA, Interferon-α and cyclosporin A with reasonable but not always convincing effect. An international randomised trial in multi-system LCH has been conducted by the Histiocyte Society aiming to evaluate the effects of VBL and VP16 administered as single agent over a period of 6 months, combined initially only with one pulse of methylprednisolone. The evaluation of disease response according to a new definition and assessment showed a clear difference between patients who did respond to the therapy within 6 weeks after start of treatment and those with stable symptoms and signs or progressing features. The recurrence rate and mortality rate in the non-responder group was high. These data have recently been confirmed convincingly by retrospectively evaluating the results of the cooperative DAL HX-83 and -90 studies in which a more aggressive initial therapy (PRED, VBL, VP16) was followed by prolonged continuation treatment (6-mercaptopurin and PRED/VBL /+/- VP16 or MTX pulses). These data strongly support the thesis that the response to the initial treatment is an important indicator of the outcome, thus facilitating the introduction of salvage therapy early in the course of resistant disease.

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EPIDEMIOLOGY OF OSTEOSARCOMA

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Primary bone tumours are rare, constituting only 0.2% of all cancers (0.8 per 100,000 population in the Western world). In large population surveys osteosarcoma is the most frequent primary malignant bone tumour, accounting for 35% of cases. Juxtacortical, surface, osteosarcomas account for 5% of all osteosarcomas. There appears to be little variation in incidence by age or sex, although there is a tendency for the onset to be slightly earlier in girls. Below the age of 20 the tumours occur in the long bones (80%) but there is a bimodal distribution of incidence, and above the age of 50, extremity cases account for only 50% and 20% of cases then occur in the pelvis. The median age of onset is higher in whites than in blacks. In the UK the relative risks for osteosarcoma are similar in Caucasian, Afro-Caribbean, and Asian children. In contrast, Afro-Caribbean children have a zero risk of Ewing's sarcoma or bilateral retinoblastoma. The second peak of incidence, over the age of 50, is in part related to Paget's disease and, at this age, the pelvis and facial bones account for 20% of tumour sites with the extremity lesions accounting for less than one-third of that in younger patients. Of the known environmental aetiological factors, radiation is by far the most important. About 1 in 5,000 patients *undergoing* therapeutic irradiation will develop a radiation sarcoma. The latent period ranges from 5-25 years, with a mean of 10 years following ionizing radiation. Radiation-induced sarcomas are always central and of high grade. If they are not metastatic, and resectable, cure is possible. Many of the sites of radiation of the primary tumour (breast and pelvis) mean that resection of the radiation-induced sarcoma is difficult or impossible. Follow up of the radium dial workers has shown a very high lifetime risk of osteosarcoma, especially in those women who ingested more than 750μCi of radiation. There is a cumulative lifetime risk with no diminution in time from exposure. Genetic factors are of great importance in the development of osteosarcoma. The risk of developing the tumour is increased 500-1000 fold in patients suffering from bilateral (hereditary) retinoblastoma. Although many of the cases occur within the radiation field the risk is greatly elevated for tumours occurring in the long bones, outside the radiation field. The risk appears to be increased by the concomitant administration of anthracyclines and alkylating agents. Children with Ewing's sarcoma are at increased risk of development of radiation-induced osteosarcoma. The latest figures from the United Kingdom suggests that this risk is approximately 7-fold higher than the risk of osteosarcoma in the general population. In spite of the fact that this is still a low risk, it is grounds for concern in the use of therapeutic radiation for Ewing's sarcoma. Reassuringly, apart from Ewing's sarcoma, there appears to be little risk of induction of osteosarcoma when therapeutic radiation is used in childhood for other diagnoses. Other genetic risks include the Li-Fraumeni syndrome (due to germ line mutation of p53) but, in sporadic cases of osteosarcoma, this appears to account for only 3-5% of cases.

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EPIDEMIOLOGY OF OSTEOSARCOMA

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MINIMAL RESIDUAL DISEASE IN CHILDHOOD ACUTE LEUKEMIA

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The majority of childhood leukemias (~85%) represents acute lymphoblastic leukemias (ALL), whereas 10-15% are acute myeloid leukemias (AML). Despite major improvements in treatment, still 25-30% of children with acute leukemia relapse, although virtually all children achieve complete remission according to cytomorphological criteria. The detection limit of cytomorphological techniques is only 10^{-1} to 10^{-2} (10 to 1 leukemic cells in 100 normal cells) and therefore limited information is provided about the effectiveness of the applied treatment. More sensitive techqniques should be used for the detection of minimal residual disease (MRD) to obtain better insight in the reduction of tumor mass during induction treatment and further eradication of the leukemic cells during maintenance treatment. Two main techniques are available for detection of MRD in acute leukemia, i.e. immunological marker analysis and the polymerase chain reaction (PCR) technique.

Immunological marker analysis allows detection of unusual and aberrant immunophenotypes, and is performed by fluorescence microscopy or, more usually, by flow cytometry. Recent developments in flow cytometry allow the application of multiparameter analysis with the simultaneous detection of two scatter parameters and three immunological markers, the combined detection of surface membrane and intracellular markers, and consecutive 'gating' steps, which focus on the leukemic cell population. Dependent on the marker combinations and the consecutive flow cytometric 'gating' steps, detection limits of 10^{-3} to 10^{-5} can be reached. Multiparameter flow cytometry allows MRD detection in 60-70% of precursor-B-ALL, more than 90% of T-ALL, and ~80% of AML.

PCR-mediated MRD techniques are based on the detection of leukemia-specific nucleotide sequences, such as junctional regions of rearranged immunoglobulin (Ig) and T cell receptor (TcR) genes or breakpoint fusion regions of chromosome aberrations. The junctional regions of rearranged Ig and TcR genes can be regarded as unique "fingerprint-like" sequences, because of the many possible combinations of variable (V) and joining (J) gene segments in each Ig/TcR gene locus and because of the deletion and random insertion of nucleotides in V-J junctional regions during the rearrangement processes. Therefore junctional regions are assumed to be different in each lymphocyte and thus also in each lymphoid leukemia. Based on this assumption, junctional regions of rearranged Ig and TcR genes are used as targets for PCR-mediated MRD detection, using primers at opposite sides of the junctional region, e.g. V and J gene-specific primers. Leukemia-specific junctional region probes have to be designed for each individual patient in order to discriminate between the leukemia-derived junctional region and junctional regions from normal cells which have rearranged the same (or comparable) V and J gene segments as the leukemic cells.

PCR analysis of junctional regions of rearranged Ig and TcR genes can be applied for MRD detection in the majority of ALL cases, because identifiable IgH gene rearrangements are found in 80-90% of precursor-B-ALL and identifiable TcR-γ and TcR-δ gene rearrangements occur in ~90% and ~50% of T-ALL, respectively. Cross-lineage TcR-γ and/or TcR-δ gene rearrangements are also found in at least 75% of precursor-B-ALL. Dependent on the type of rearrangement and the size and composition of the junctional region, MRD can be detected down to 10^{-3} to 10^{-6}. However, one should be aware that changes in Ig/TcR gene rearrangement patterns during follow-up will cause false negative MRD results.

For MRD-PCR studies of chromosome aberrations primers are designed at opposite sides of the breakpoint recombination area so that the PCR product contains leukemia-specific fusion sequences. If the breakpoints cluster in a small area, the PCR analysis can be performed at the DNA level, such as in the T-ALL associated aberrations t(1;14)(p34;q11), t(10;14)(q24;q11) and the site-specific deletions in the *SIL/TAL1* gene region on chromosome 1, the so-called *TAL1* deletions. In most translocations the breakpoints are spread over much larger areas, but frequently a new leukemia-specific fusion gene is created, which is transcribed into a leukemia-specific fusion mRNA. This fusion mRNA can be used as RT-PCR target for detection of MRD. Examples are: *E2A-PBX1* mRNA in pre-B-ALL with t(1;19)(q23;p13), *TEL-AML1* mRNA in $CD10^+$ precursor-B-ALL with t(12;21)(p13;q22), *PML-RARA* mRNA in AML with t(15;17)(q23;q21), and *AML1-ETO* mRNA in AML with t(8;21)(q22;q22).

The advantage of using specific chromosome aberrations as leukemia-specific markers is their stability during the disease course. However, only 30-35% of childhood ALL and 25-40% of AML have a specific chromosome aberration. In addition, one should be aware that in many laboratories cross-contamination of RT-PCR products has shown to be the major cause of false positive results.

The preliminary results of immunophenotypic and molecular MRD studies in precursor-B-ALL, T-ALL, AML-M3 with t(15;17), and AML-M2 with t(8;21) show that MRD detection is valuable to get insight in the effectiveness of leukemia treatment. However, these studies also indicate that the meaning of MRD positivity might be different in each type of acute leukemia. Therefore large scale prospective studies are needed to establish the prognostic value of MRD detection and to determine whether MRD information can be used for adaptation of remission criteria and for stratification of treatment protocols. Furthermore, one should be aware that each MRD technique has its advantages and limitations, which have to be weighed carefully to make an appropriate choice. In addition, standardization of the MRD techniques and design of optimal MRD strategies are needed before they are used for stratification or adaptation of treatment protocols.

Both authors are members of the BIOMED-1 Concerted Action entitled "*Investigation of minimal residual disease in acute leukemia: International standardization and clinical evaluation*".

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THE RESPONSE OF NORMAL LYMPHOCYTES TO ANTI-LEUKEMIC DRUGS A NEW REFERENCE SYSTEM FOR THE IN-VITRO MTT-TEST

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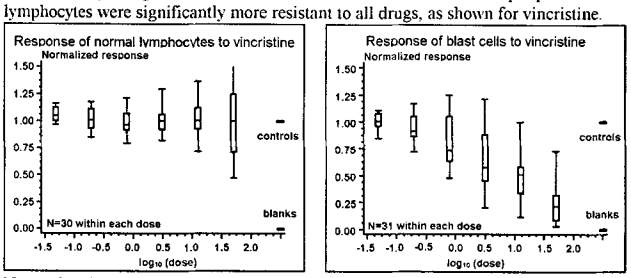
The methyl - thiazol - tetrazolium (MTT) assay has been frequently used to assess resistance of blast cells against anti-leukemic drugs in vitro. As of yet the results have been of limited use due to their low positive and negative predictive value. To this end peripheral lymphocyte samples of 30 healthy persons and leukemic blast cell samples of 31 patients were tested against a panel of 16 anti-leukemic drugs. Cells were exposed to six, exponentially increasing doses of each single drug for 72 h in triplicate. Intra-experimental control was carried out by culturing cells of the same sample without drugs (controls) as well as blanks. Response to drug exposure was normalized by using controls and blanks as reference values. Normal peripheral blood lymphocytes were significantly more resistant to all drugs, as shown for vincristine.



Normative data were defined from the responses of normal lymphocytes for each drug by computing the area under the dose - response - curve (AUC) of the fifth percentiles, see lower whiskers of the left figure. The ratio of AUCs of blast cells and normative data results in an index of the effectiveness of a given drug to kill blast cells of an individual patient. An index > 1 indicates that a drug is more effective in killing blast cells than in killing normal lymphocytes. In case of vincristine this index ranged from 0.6 to 2, mean 1.1. Currently these results are used in case of toxicity related delay of anti-leukemic therapy. That is to decide which drug should be reduced first.

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P-2

SIGNIFICANCE OF IMMUNOGLOBULIN LEVELS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: A REPORT OF THE CHILDRENS CANCER GROUP (CCG)

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Disturbances in immunoglobulin(Ig) levels have been described in patients with leukemia since the 1960's. Different suppression patterns have been reported secondary to small numbers of patients and various treatment protocols used. The etiology of altered Ig levels and their significance has not been well delineated. We report on 622 children with ALL treated on CCG protocol 1922. Pts were randomized to receive decadron (D) [6mg/m2/d] vrs prednisone (P) [40mg/m2/d] during induction (IND); followed by a 3 mo consolidation (CON) with randomization to 11 weekly doses of IV 6MP (I) [1gm/m2/wk] or oral 6MP (O) [75mg/m2/d]; followed by a 2 mo delayed intensification (INT) containing D; followed by 3 month maintenance cycles (MAINT) with oral 6MP and monthly 5 day pulses of D or P. Ig levels were drawn at the start of each phase of therapy and at the start of each maintenance cycle until normal. Very few patients received Ig infusions(<2%) - these pts were eliminated from analysis. At diagnosis, 35% of the patients had at least one low Ig level (<2 S.D. for age), by MAINT cycle 1 this number rose to 87%. Patients treated with D were more likely to develop panhypo-Ig (CON, OR=3.06, p=0.002) as were patients treated with IV 6MP (INT, OR=3.42, p=0.003). We found little evidence that the occurrence of serious infections or sepsis was assoicated with depressed Ig levels, except perhaps for Hypo-IgG in CON. Using life table analysis, hypo-IgG in induction portended a poorer EFS (OR=3.26, p=0.05). There were no differences yet in EFS for hypo-IgA, M or panhypoIg, but longer follow-up is required for more leukemic events to occurr. From this analysis we can conclude that D and I may be more immunosuppressive than P and O respectively. Hypo-IgG may be an important prognostic indicator in childhood ALL.

P-3

DETECTION OF MINIMAL RESIDUAL DISEASE (MRD) IN ACUTE LYMPHOBLASTIC LEUKEMIA(ALL) BY AMPLIFICATION OF THE CDR3 REGION OF THE IMMUNE GLOBULIN HEAVY CHAIN GENE: COMPARISON OF ALTERNATIVE METHODS.

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Background: PCR of IgH and TCR gene rearrangements are useful for detection of MRD in ALL. Synthesis of clone specific oligonucleotide probes (OL) appears to be the most sensitive and specific approach. Simple techniques that obviate the need for sequencing would be highly useful provided they retain sensitivity and specificity. **Objectives**: to compare two methods of MRD detection by amplification of the CDR3 region of the IgH gene in ALL. **Methods**: DNA from 32 newly diagnosed pts. with B cell ALL was amplified with consensus primers for each VH gene family and JH. Positive PCR products were cut with HaeIII and used as a direct DNA probe (PR). The CDR3 region of these products was sequenced and OL were derived from NDN sequences, preferentially of the DNJ border. Both PR and OL were used to hybridize amplified DNA from diagnosis and sequential remission bone marrow samples from the same patients obtained every three months, as well as PBL controls. Follow up samples were amplified with FR2-JH primers and PCR products were hybridized by Southern blot. **Results**: 88 DNA samples were analyzed with both techniques. Positive hybridization with OL was seen one to 25 months after start of therapy. Sensitivity of PR detection was 97% and specificity was 50% compared to OL. 42% of PR hybridized to PBL vs 13% of OL. **Conclusion**: Sequencing of CDR3 and synthesis of derived oligos appear as a necessary step to obtain specific detection of MRD in ALL.

P-4

ANALYSIS OF THE COMPLEMENTARY DETERMINING REGION III (CDR3) OF THE IMMUNOGLOBULIN(Ig) HEAVY CHAIN LOCUS IN ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILEAN CHILDREN.

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Background:ALL occurs in early lymphocytes at the time of Ig rearrangment. Analysis of these rearrangements could identify distinct subsets of lymphocytes prone to transformation. **Objectives:** To analyze the sequence of the CDR3 region of the IgH gene in ALL in chilean children. **Methods:** We studied 40 VDJ rearrangements from 32 unselected children with precursor B cell ALL from Chile at diagnosis. Rearrangements were derived by PCR with VH gene family specific primers and sequenced directly. **Results**: Multiple VDJ rearrangements were seen in 28% of cases. VH gene family usage was non random with excess usage of VH 4, 5 and 6. DH was representative of family size; tandem DH genes appared in 22% of cases. JH gene usage was biased with predominance of JH4 and JH6. These data coincide with previous reports from developed countries (Eur. J. Immunol 1990; 20: 2209, Eur J Immunol 1994;24:900). CDR III regions represented an unbiased repertoire; VH to JH joinings were in frame in 36% of cases. Absent N nucleotides in the DJ border, suggestive of fetal origin of ALL, were seen in 9/40 rearrangements but they did not correlate with younger age . More than one rearrangement was sequenced in 6 patients, representing independent events with no signs of clonal evolution. One patient was analysed at first bone marrow relapse showing persistence of one rearrangement and evolution of a second one which conserved the DJ border. **Conclusion**: The subset of B cell precursors which suffer malignant transformation to ALL appear to be common in different parts of the world.

P-5

STUDY OF CHEMOTHERAPY - INDUCED APOPTOSIS IN PERIPHERAL BLOOD OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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In acute lymphoblastic leukemia (ALL) the apoptosis of blast cells in peripheral blood (PB) and bone marrow (BM) before and/or during the treatment, is of great interest. We studied apoptosis before and during induction therapy, directly in 146 PB samples of 22 children with ALL at diagnosis and one at relapse. The apoptotic index of the percentage of circulating apoptotic cells (CAC) of the cases at diagnosis, indicated maximum apoptosis 48 h after the onset of treatment followed by a decrease at 72 h. After 96 hours of treatment they were distinguished in three groups: **a)** with negligible CAC (12 cases, 55%), indicated a rapid and successful apoptotic action of treatment. Immunocytochemical analysis in BM and PB diagnosis samples showed that all cases were negative for mdr-1 overexpression, while 7 of them overexperssed p53, **b)** with CAC between 8% and 12% (7 cases, 32%), indicated a more extensive apoptotic action of treatment. Immunocytochemicaly all these cases were negative for mdr-1 as well as for p53 overexpression and **c)** with extremely high percentage of CAC, over 24% (3 cases, two of which died, never entered in remission), all negative for mdr-1 and p53 overexperession. In the case of relapse, characterized by strong mdr-1 overexpression and negative p53, the CAC remained negligible at all time intervals, because of the resistance of blast cells to chemotherapy. These results indicate that, it is possible to evaluate the response to the treatment by the study of apoptosis in PB. Thus the study of apoptosis in PB together with molecular events, will be a prognostic factor in the treatment of ALL.

P-6

IS A HIGH ACTIVITY OF FOLYLPOLYGLUTAMATE HYDROLASE (FPGH) A MECHANISM OF METHOTREXATE RESISTANCE IN CHILDHOOD LEUKEMIA?

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Methotrexate resistance can be caused by: a) impaired membrane transport, b) impaired polyglutamation and c) increased or altered levels of target enzymes. Methotrexate (MTX) is effective in acute lymphoblastic leukemia (ALL) but not in acute myeloid leukemia (AML). Intrinsic resistance to MTX in AML is suggested to be caused by an inefficient polyglutamation. This polyglutamation depends on the balance between formation of polyglutamates catalyzed by folylpolyglutamate synthetase (FPGS) and the breakdown of these products by folylpolyglutamate hydrolase (FPGH). In this study we analyzed whether differences in MTX sensitivity between childhood ALL and AML could be detected in vitro and whether AML and ALL cells differ in their FPGH activities. ***Methods***: In vitro MTX sensitivity was determined by the inhibition of the folate requiring enzyme thymidylate synthase (TS) after 3 hrs of incubation with this drug followed by a 18 hr drug-free incubation period. FPGH activity was studied with MTX-Glu_2 as substrate. ***Results***: The IC_{50} value of MTX for 40 untreated ALL and the 9 relapsed ALL cases tested did not differ: 1.28 μM (range <0.16 to >40) vs 1.22 μM (range 0.71 to 4.71). The IC_{50} was 7-fold higher for the 12 AML cases tested: 8.13 μM (range 0.1 to >40). The FPGH activity was median 2-fold higher in 9 AML cases (1.16 nmol/hr/10^6 cells; range 0.42 to 25.46) than in 37 untreated ALL cases (0.54; range 0.01 to 28.90). However, in the 23 cases in which both the IC_{50} value for MTX and the FPGH activity were measured we could not not reveal a high correlation between these two factors. ***Conclusion***: MTX resistance can be determined in leukemic patients with the TS inhibition assay. A high FPGH activity might partly explain the intrinsic MTX resistance in childhood AML. Other factors play an important role in determining MTX resistance in childhood leukemia.

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P-7

DETECTION OF MLL/AF4 AND BCR/ABL REARRANGEMENTS AND MONITORING OF MINIMAL RESIDUAL DISEASE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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We used reverse transcriptase - polymerase chain reaction (RT-PCR) for detection of BCR/ABL and MLL/AF4 rearrangements, molecular hallmarks of t(9;22) and t(4;11) in pediatric patients with acute lymphoblastic leukemia (ALL). We found 5 out of 130 newly diagnosed or relapsed B-cell precursor ALL positive for the presence of BCR/ABL fusion transcript. Two of them underwent allogeneic bone marrow transplantation (BMT) from a sibling donor and 3 were treated with chemotherapy alone. We analyzed 24 remission samples for detection of minimal residual disease (MRD). One patient lacked evidence of MRD by RT-PCR 21 months after BMT. In contrast, 4 children exhibited BCR/ABL transcript in complete remission (CR), 2 of them in all remission samples tested. We also demonstrate our effort to eradicate BCR/ABL positive clone from hematopoietic progenitor cells using ex-vivo purging with etoposide and methylprednisolon. Five infants with ALL and 1 ten-year old patient with cytogenetically proven t(4;11) were examined for the presence of MLL/AF4 chimeric gene. Three out of 5 infants and the patient with t(4;11) exhibited MLL/AF4 in diagnosis sample. Two infants did not reach CR and died early in the course of treatment. The third MLL/AF4 positive infant achieved molecular remission after remission-induction therapy. Unfortunately, MLL/AF4 transcript appeared again during intense chemotherapy and, subsequently, patient relapsed and died. In this case, we were able to predict sudden and unexpected relapse prior to cytogenetic methods, including fluorescence in-situ hybridization (FISH). We demonstrate the precision of RT-PCR in monitoring of MRD in patients with gene rearrangements.

P-8

IMMUNOPHENOTYPIC AND CYTOGENETIC CHARACTERISTICS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN THE SCHNEIDER CHILDREN'S MEDICAL CENTER OF ISRAEL (SCMCI)

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Between 1984-95, 186 newly diagnosed children with ALL enrolled in the SCMCI in two Israeli National Studies: INS'84-73 pts, INS'89-113 pts constituting 60% and 40% respectively, of Israeli childhood ALL. (INS'89 is an Israeli modification of ALL-BFM-86 protocol). A total of 86% and 75% of the pts were Jewish; the remainder were Moslem, Bedouin and Christian. T-cell phenotype was about 30%, CALLA+ in 43%, CyIg+ in 21%. Cytogenetic studies were performed on 52 and 97 pts of the INS'84 and '89 protocols of them 36% and 88% were successful respectively. Cytogenetic results are shown in the table:

	INS'84 -19pts	INS'89 -85pts
Normal	58%	30.5%
Pseudodiploid	15%	30.5%
Hypodiploid	5%	2.3%
Hyperdiploid (<50)	5%	16.4%
" (>50)	16%	15.3%
" (>65)	-	4.7%

In the later protocol, t(1:19) and t(17:19) were detected in 3 pts and 1 pt respectively, t(9:22),t(4:11), dic(9:12) in 2 pts each, all were associated with B lineage phenotype as well as all the hyperdiploid (50-60).t(14q11) were detected in 8 pts, all associated with T-cell lineage; del(6q) in 10 pts was associated in 6 of them with T lineage; del(9p) was found in 6 pts. Regarding ethnic distribution, Jewish pts predominated the hyperdiploid (55-65) group (11/13) and non-Jewish pts the 47-48 chromosones group (8/13).The overall 3 years event-free survival at the SCMCI for the '84 and '89 protocols with a median follow-up of 9 and 3 yrs is 72% and 76% respectively. The epidemiological aspects, and clinical relevance of the cytogenetic and immunophenotypic characteristics of childhood ALL in the SCMCI will be further analyzed.

P-9

AUTOLOGOUS CYTOTOXICITY RELATED TO HLA CLASS I AND ADHESION MOLECULE EXPRESSION ON ACUTE LEUKEMIC BLASTS IN AN IN VITRO CO-CULTURE SYSTEM

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Purpose: To evaluate specific autologous cytotoxic activity and the relationship to the expression of HLA class I and adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) on the appropriate leukemic cells.
Methods: Mononuclear blood cells (MNC) of 9 patients (pts) in complete remission and off-therapy (2 pre-pre B-ALL, 1 pre B-ALL, 4 c-ALL, 1 T-ALL, 1 AMMoL) were co-cultured for 2-3 weeks in medium with 10% AB serum and 10^2 U/ml natural interleukin-2 (nIL-2) with autologous 40Gy-irradiated cryopreserved blasts. Cytotoxicity of effector cells was tested after 2 and 3 weeks (2 pts were only tested once) against native autologous blasts and after immunological blocking of HLA class I receptor in a 4h ^{51}Cr release assay. Leukemic cells were immunologically characterised for HLA class I, CD54 and CD58 expression by FACScan. Cytotoxicity of effector cells was related to the percentage of positive cells and to the intensity of molecule expression on targets.
Results: Effector cells generated in the co-culture system revealed a median lysis of autologous target cells of 9.5% (n = 16, min. 2.4%, max. 56.5%). HLA class I receptor blockade of these targets resulted in a significantly decreased cytotoxicity of 1.15% (n = 12, min. 0%, max. 21.6%, p = 0.02). There was, however, neither a significant correlation between the percentage (29.8% - 96.3%) of positive target cells nor the intensity of HLA class I expression to the cytotoxic activity. This was also true for the expression of CD54. In contrast, intensity of CD58 expression was significantly positively correlated to the cytotoxic activity (r = 0.83, p = 0.02). In general, a trend of positive correlation was seen between the intensity of molecule expression per target cell and the cytotoxicity.
Conclusions: Effector cells generated in an autologous lymphocyte-blast culture system revealed a distinct antileukemic activity depending on HLA class I-restricted T-cells. Furthermore, cytotoxicity is strongly influenced by the expression of CD58, a molecule which enables cell to cell attachment by interacting with its ligand CD2. At the same time this interaction leads to T-cell as well as NK-cell activation. Thus, it has to be considered that generation of tumor-specific lymphocytes in vivo (e.g. vaccination) may be limited due to a lack of expression of adhesion molecules. IL-2 could upregulate the production of cytokines (TNF-α) which positively influence adhesion molecule expression.

P-10

THE EVIDENCE OF THE FAS/APO-1 ANTIGEN ON THE BLAST CELLS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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There has been studied the expression of FAS/Apo-1 antigen, which mediats apoptosis, in the blast cells of the 27 children with ALL. Cells from peripheral blood (PB) and bone marrow (BM) were investigated before any courses of the chemotherapy. Blast cells of patients were immunophenotyped with the help of monoclonal antibodies (MAbs) against CD2, CD3, CD5, CD7, CD10,CD-11b, CD19, CD22 & HLA-DR antigens. Cytoplasmic immunoglobulins were also studied. FAS/ Apo-1 antigen expression was revealed by MAbs IPO-4 with the help of indirect surface immunofluorescence on flow cytofluorimeter FACScan and was found in 14 patients (pts) from 27 (50%).According to this methods all patients were divided into 5 subgroups and in each subgroup the number of patients (pts) with expression FAS+ antigen on BM blasts was noted: pro-B-ALL - 2/7 pts, pre-pre-B-ALL - 8/11 pts, pre-B-ALL - 1/2 pts, T-ALL - 2/5 pts and "null"-ALL - 1/2 pts. Pre-pre-B-ALL clinically is the most favourable variant of ALL. It represents the earliest stages of B-cell differentiation, and has the most close correlation between antigens CD-10 and CD-95. Compared 2 groups (FAS+ and FAS- in BM cells) we have noticed significantly($p < 0.01$) higher survival (8 - 10 yrs follow up) in group with FAS+ blasts.

P-11

EXPRESSION OF P-GLYCOPROTEIN IN CHILDREN'S AND ADULTS' HEMATOLOGIC MALIGNANCIES

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Drug resistance has been shown to be associated with the expression of P-glycoprotein (P-gp) the product of mdr-1 gene. In the present study expression of P-gp was assessed on fresh frozen sections and cytospins prepared from peripheral blood mononuclear cells (PBMC) obtained from leukemic patients and separated by Ficoll gradient centrifugation and three antibodies (Abs) directed to three different epitopes of P-gp molecule: C219, JSB-1, MRK16 and immunohistochemical APAAP staining method. We consider positive for P-gp if they were positive with at least two Mab's. Lymph node biopsies were surgically resected from 57 patients with untreated non-Hodgkin's lymphoma (NHL). They consist of: 40 low grade and 17 high grade, as well as from 6 reactive lymphoid tissue (RLT). Seventy eight leukemic patients: 45 children, and 33 adults samples of PBMC were also examined. Human myelogenous leukemia cell line K562 and Vincristine resistant K562VCR served as a negative and positive controls for reactivity of three Abs, respectively. Staining for P-gp was noticed in 1 of 10 CLL, 2 of 2 HCl, 0 of 9 IC, 2 of 12 CB/CC, 1 of 7 CC low grade and in 3 of 10 CB, 0 of 2 IB and 0 of 5 LB high grade NHL. There was not found any reactivity of three Abs in reactive lymphocytes. Weak to moderate staining of 10-50% cells was found on high endothelial venules as well as in macrophages in RLT and NHL. In NHL biopsies pattern of immunoreactivity was heterogeneous ranging from a high frequency of positive cells up to 50%, to only very few scattered positive cells. In children's leukemia samples positive for P-gp staining was observed in 8 of 29 ALL, 3 of 9 AML, 1 of 1 CML and 2 of 6 NHL, in adults' in 2 of 6 ALL, 1 of 1 CML and 8 of 26 AML samples. Positive staining for p-gp was observed in 15% and 17.6% low and high grade NHL, respectively. In adults' and children's leukemia samples staining for P-gp was observed in 31% and 33%, respectively. We have not found any correlation between positive for P-gp staining and clinical parameters.

P-12

DETERMINATION OF CLONAL CHARACTER OF B LINEAGE CHILDHOOD ALL BY PCR.

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About 80% of ALL are due to clonal expansion of B-cell precursors and show gene rearrangements in the heavy chain immunoglobulin (IgH) resulting in regions relatively conserved called frameworks and 3 regions hypervariable known as complementarity determining regions (CDR). One of them, CDR3, is unique in each B-lineage cells and can be used as clonal marker to B-lineage leukemia. T-cell receptor (TCR) rearrangements have also been found in ALL of B-lineage , principaly incomplete rearrangemnts of TCRδ, as Vδ2-Dδ3. These sequense can be amplified by PCR using consensus primers to the regions CDR3 and TCRδ. We analysed by PCR using consensus primers to the conserved regions that flank CDR3 and TCRδ the detection of clonality in 53 pediatric patient: 36 with B-lineage ALL, 7 with T-lineage ALL, 4 with acute non lymphobastic leukemia, 1 with Hodgkin lymphoma, 2 with idiopathic thrombocytopenic purpura, 2 with aplastic anemia and 1 with pure red cell aplasia. Clonality by PCR was found in 86,1% of patients with B-lineage ALL when used primers to the CDR3 region, in 41,6% when used primers to TCRδ and in 91,6% when used both of them. Clonality was not found in other cases. Concluding, PCR was able to detect clonality in 91,6% of patients with B-lineage ALL and proved to be a specific method to detection of malignancies of B-lineage, showing advantages when compared to other molecular analyses methods as Southern blot.

P-13

THE P-GLICOPROTEIN (P-170) AS EXPRESSION OF THE MULTIDRUG RESISTENCE (MDR), ANALYZED WITH C-219, M M 4.17 E JSB1 IN CHILDREN WITH ACUTE LEUKEMIA

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The phenotype MDR is combined with the overexpression of a membrane glycoprotein (P-170) which is codified by MDR 1 gene responsible for the expulsion of cytostatic from malignant cells. The aim of our research was to evaluate the expression of the P-170 using three monoclonal antibodies in children with AL.
The leukemia blasts of 64 patients have been examined: 12 LAM, 14 LAL-T and 38 B-LAL, 19 38 with co-expression of one or more myeloid antigens (My+), followed between January 1992 and January 1996. 10/12 LAM have been studied at onset and two at relapse; 13/14 T-LAL at diagnosis and one at relapse. 15/19 B-LAL My+ have been evaluated at onset and 5 at relapse; finally 14/19 B-LAL My- at diagnosis and 5 at relapse. The protein P-170 has been examinated using three monoclonal antibodies with immunocytochemistry method (LSAB plus alkalyne phosphatase as revealing system). The antibodies C-219 and JSB1 react with a conserved cytoplasmatic epitope of P-170 which rapresents the functional portion of the protein, while MM4.17 recognizes a surface epitope strongly conserved of P-170. We classified those cases with 2% or more positive cells as being positive cases.
In the three series of patients (pts) the percentage of MDR positivity is 42 (22/52) at diagnosis and 83 (10/12) at relapse. In LAM group 6 pts (60%) are P-170 positive at diagnosis, 2/6 are in complete remission (CR) and one is too early. Of the 13 T LAL, 6 are MDR positive and two are in RC. In B LAL My+ group 7/16 (46%) are MDR positive at diagnosis,of them 6 are in RC as 7/8 P-170 *negative at diagnosis. Of the* 14 B LAL My-, 3 are P-170 positives at diagnosis (21%), all in RC as 9/11 P-170 negative. These results allow us the following considerations: 1) High percentage of P-170 positivity at bone marrow relapse, 10/12 (83%), of them 6 positives at diagnosis too. 2) High P-170 positivity at diagnosis in LAM group (60%), as well known in adult leukemia. 3) P-170 is more expressed in B-LAL My+ versus B-LAL My- at diagnosis (46% vs 21%). Whereas P-170 positivity doesn't seem to worsen the prognosis in B-LAL My+ patients. More data and longer follow up are needed for further evaluation.

P-14

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) WITH SEVERE SKELETAL INVOLVEMENT - A SUBSET OF CHILDHOOD ALL CHARACTERIZED BY HIGH DNA-INDEX (DI) AND GOOD PROGNOSIS

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Skeletal radiographic abnormalities are common in children with ALL. The impact of severe skeletal involvement (SI) on survival and the correlation between SI and immunological and cytogenetical markers were analyzed. 106 ALL pts (62 m, 44 f, aged 0.6 to 17.8 yr) were treated at the University Children's Hospital Würzburg between 1974 and 1995 according to the BFM protocols. 59 pts (55%) showed radiographic abnormalities as metaphyseal banding (48%), periosteal reactions (11%), osteolysis (33%), osteosclerosis (31%) and osteopenia (22%). Moderate SI was defined as a presentation of 1 to 4 lesions, severe SI as a presentation of more than 4 lesions. Differences in regard to radiographic abnormalities were: a higher rate of geographic osteolysis (37%; $p<0.001$) and periostal reactions (28%; $p<0.05$) in pts with severe SI (n=32) than in pts with moderate SI (0% and 4%, respectively). Pts with severe SI showed a lower peripheral blast count ($p<0.05$) at diagnosis, more frequent a 'prednisone good response' ($p<0.05$) and a higher survival rate (83±7%; $p<0.05$) than pts without SI (54±9%). However, no differences were found in terms of organ size of spleen and liver and BFM risk factor. Pts with moderate SI showed a higher hemoglobin concentration ($p<0.05$), an increased size of liver ($p<0.05$) and spleen ($p<0.01$), a higher BFM risk factor ($p<0.01$), but still a higher survival rate (73±11%) than pts without SI. Pts with severe SI had a significantly higher ($p<0.001$) DNA content of leukemic cells as measured by DNA-index (DI) than pts without SI. 31% of pts with severe SI, 22% of pts with moderate SI and no pt without SI had a DI≥1.16. No pt with a DI<1.0 presented with a severe SI. The degree of SI was not related to immunological markers, age at diagnosis, sex, duration of history, CNS involvement or parameters of bone metabolism. We conclude that based on clinical features two distinct subgroups could be identified in terms of SI. Pts with clinically relevant and severe SI had a better prognosis than pts without any SI. The number of radiographic abnormalities in pts with SI correlated with the DI (rho:0.46; $p<0.001$). Therefore, the favorable outcome of pts with severe SI can be explained by the higher DNA-index in this subgroup.

P-15

COMPARISON OF THE CELLULARITY AND PRESENCE OF RESIDUAL LEUKEMIA IN BONE MARROW ASPIRATE AND BIOPSY SPECIMENS IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AT DAY 7-14 OF CHEMOTHERAPY

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In pediatric patients with ALL where the treatment protocol requires a bone marrow evaluation 7-14 days after the initiation of chemotherapy, the question of the need for a bone marrow biopsy, in addition to an aspirate, is frequently raised. This study addresses the evaluation of bone marrow cellularity and presence of residual leukemia in both aspirate and biopsy specimens in 45 consecutive pediatric patients (ages 1-19 years, 19 females and 26 males) with ALL 7-14 days after initiation of therapy. Forty-three patients were receiving initial induction chemotherapy for ALL; 2 were being treated for bone marrow relapse. 20/45 patients showed evidence of residual leukemia by bone marrow biopsy; 16/20 (80%) of these had evidence of residual leukemia in the aspirate specimen. Of the 4 aspirate specimens that did not demonstrate residual leukemia, 2 had <5% blasts and 2 had too few cells in the aspirate for evaluation. Of the 25/45 bone marrow biopsy specimens with no detectable residual leukemia, 14 of the aspirates had <5% blasts, and 11 had too few cells in the aspirate for evaluation. 13/45 (29%) of the aspirates had too few cells for a differential count. The bone marrow cellularity judged from the aspirate specimen was considered to be low (0-1+) in 34/45 patients. Of these 34 patients, the bone marrow biopsy showed hypocellularity (<20% cellularity) in 12/34, moderate cellularity (20-79% cellularity) in 14/34, and hypercellularity (>79% cellularity) in 8/34. We conclude that both the bone marrow aspirate and biopsy specimens provide important information in evaluating the response to chemotherapy in pediatric patients with ALL at day 7-14 of chemotherapy. The aspirate alone may be misleading in terms of cellularity in many patients and may not provide evidence of residual leukemia in up to 20% of patients.

P-16

A NEW PROTOCOL FOR TREATMENT OF RELAPSED CHILDHOOD ALL
ALL - REZ BFM 95: CONCEPT AND FIRST RESULTS

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Even though 70 - 80% of children presenting with initial acute lymphoblastic leukemia (ALL) can be cured, the others suffer a relapse. Currently their chances to be cured are as low as 30 - 40%. Therefore, the Berlin - Frankfurt - Münster (BFM) study group seeks to improve the prognosis for children with relapsed ALL in multi-centre trials since 1983. The most recent, ALL - REZ BFM 95, started July 1st, 1995. The characteristics are:

- New stratification into 4 groups, S1 - S4, depending on time point of relapse (very early, early, late), location (bone marrow, bone marrow + other site, extramedullary), and immunophenotype (non - T, (pre-) T)
- Increased dose intensity during induction by calculated reduction of specific drugs instead of delaying entire therapy blocks in case of severe toxicity
- Reinduction pulses during maintenance for standard risk patients, group S2
- Reduction of toxicity in children with poor prognosis, group S4
- bone marrow transplantation for all high-risk patients, groups S3 and S4
- New insights in pathology of ALL by scientific investigations

Among both groups S2 and S3 the application of G-CSF is randomized to assess its value for therapy of relapsed ALL.

As of Feb. 1st 1996 54 patients were treated according to the protocol. Forty-four fulfilled all inclusion criteria, 3 were assigned to group S1, 16 to S2, 9 to S3, and 16 to S4. Response to therapy could be determined in 15 patients, only 2 did not respond to therapy at all. One patient of the poor prognosis group S4 suffered a second relapse, five patients died due to therapy related causes i.e. therapy refractory infections. At the conference we will be able to present the first valid estimate of the toxicity (WHO scores) of each therapy element as well as a valid estimate of the response rates.

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P-17

VERTEBRAL COLUMN INVOLVEMENT AS A FIRST MANIFESTATION OF CHILDHOOD LEUKEMIA.

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Bone pain and radiological changes are well-recognized presenting features of childhood leukemia (ALL).There is, however, agreement that the spine is less commonly involved at the time of diagnosis than are the long bones.Spinal osteoporosis leading to vertebral compression fractures is a very rare complication. Because the presenting symptoms in childhood ALL patients with vertebral compression fracture are generally atypical, the diagnosis of leukemia can be delayed.

We reported the clinical features of 23 children with vertebral compression fractures associated with ALL, who were treated between 1970 -1994 in seven hematological centers of Polish Leukemia / Lymphoma Study Group.

Between 1970 - 1981 the Memphis protocol, and between 1980-1994 the BFM protocol were used .

The duration of initial symptoms ranged from 2 to 25 weeeks. Nineteen children had back and leg pain as their initial chief complaint . Twelve of them were unable to walk because of intensive pain. Nineteen of twenty three children had multiple vertebral compression fracture and four had extensive spinal osteoporosis.

The most often vertebral fractures were localised at thoracic (Th 7-10) and lumbal (L 1-3) . Complete ALL remission (CR) and recovery from vertebral compression fracture were achieved in 21 among 23 children. 2 children died.

We found that ALL children with vertebral compression fractures usually have favorable presenting features for leukemia and that they have a relatively good treatment outcome, comparable to that of others with " standard-risk" leukemia.No mediastinal mass, no central nervous system involvement and low median leucocytes counts were seen in this group.

P-18

RANDOMIZATION OF IDARUBICINE VERSUS DAUNORUBICINE FOR INDUCTION TREATMENT IN INTERMEDIATE RISK ACUTE LYMPHOBLASTIC LEUKEMIA OF CHILDHOOD : A FRALLE STUDY.

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INTRODUCTION : From 6/93 to 12/95, 557 pts were included in protocol FRALLE 93. Among them 276 were treated in the intermediate risk group as follows : after a prophase with prednisone (P) 60 mg/m^2 x 7 d, induction was : P : 40 mg/m^2 x 15d, Vincristin 1,5 mg/m^2 x 4 (D8, D15, D22, D29), Asparaginase : 10,000 UI/m^2 x 6 (D22 to D35) ; patients were randomized in 2 arms : idarubicine (IDR) 8 mg/m^2 x 2 versus daunorubicin (DNR) 40 mg/m^2 x 2 (D8, D15). A 3rd injection of anthracyclin was mandatory in patients with more than 5% blasts on D21 marrow. 2 triple intrathecal injections were given (D1, D15). Both arms were equally balanced with 139 pts in IDR arm and 137 in DNR arm.

RESULTS :	**IDR**	**DNR**	**p**
D21 BM with > 5% blasts	13/129	22/133	0,18
(3rd injection of IDR/DNR)	13/13	19/22	
CR rate	99%	98%	NS
PNN < 500/mm^3	27 d	19,5 d	0,001
Platelets < 50,000/mm^3	12,5 d	6 d	0,001
Red cell transfusions	3,4	2,6	0,023
Platelets transfusions	3,8	2	0,005
WHO grade ≥ 3 infections	16	15	NS

CONCLUSIONS : 1) The overall CR rate is 98,5%. 2) Idarubicin is an active drug in childhood ALL. 3) After 2 anthracyclin injections there is a trend for a more effective blast killing in IDR arm. A longer FU is necessary to compare DFS in both arms as there are only 2 relapses (both in DNR arm) at the time of this analysis 4) Treatment with IDR is statistically associated with a prolonged hematological toxicity in this weekly schedule ; this may be due to the pharmacokinetics of IDR or its active metabolites. Nevertheless there is neither increase in severe infection rate nor delay in the achievement of CR in IDR arm.

P-19

PROBLEMS IN TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOMA IN CHILDREN WITH ATAXIA TELANGIECTASIA (AT).

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AT is a progressive neurological disorder with an increased predisposition to lymphoblastic leukemia and lymphoma and with an unusual sensitivity to the effects of ionizing radiation. Reports also suggest increased sensitivity to various chemo-therapeutic agents. Together with their genetic immunodeficiency, this sensitivity makes the treatment of cancer in these children a great challenge. The AT syndrome is progressively disabling and the life-span is shortened. We had to take all these facts into consideration when we planned and modified the treatment schemes.
Out of a total of 9 individuals with AT in Norway at that time, we diagnosed and treated 4 patients for cancer in 1986 - 89; 2 with T-ALL and 2 with highly malignant B-cell lymphoma. And in 1995, we diagnosed a new T-ALL among 7 living AT patients. The most surprising observation was the achievement and duration of remission in these patients after this minimized treatment.
Patient A, girl 14 yrs, had her lymphoma in a tonsil surgically removed in 1986, no futher treatment. Stayed relapsefree. In autumn 93 she developed a second malignancy, a liver tumor, and died in Dec.93.
Patient B, girl 13 1/2 yrs, had her lymphoma in the ileocoecal region removed in 1987, and then a 4 week treatment with prednisolon. No other chemotherapy and still relapsefree.
Patient C, boy 7 yrs with T-ALL in 1987,complete remission on a modified standard risk treatment. Severe abdominal and muscular side-effects of vincristin, otherwise uncomplicated until CNS- relapse one year later. Then treated exclusively with methotrexate intrathecally. No irradiaton and still in remission.
Patient D, girl 6 yrs with T-ALL in 1989, started treatment on our conventional high risk protocol. Her AT diagnosis was yet not established and she developed rapidly a marked neuro-muscular affection. Her treatment was modified. Complete remission acheived, but she relapsed when the maintenance therapy was discontinued in May 92. Tolerating even less chemotherapy at this stage, she did not achive remission and she died in Nov.92.
Patient E, girl 2 1/2 yrs with T-ALL in 1995. AT diagnosed at 2 yrs. Started on a modified treatment sceme which she tolerated much better than the others. Complete remission achieved. Still on treatment.
These case reports may indicate a possibility of treatment in AT patients with modified therapy regimen, with a fraction of ordinary doses for ALL patients. This can be associated with an increased vulnerability in all AT cells for some of the chemotherapeutic agents.

P-20

BONE MINERALIZATION AFTER (TREATMENT FOR) ACUTE LYMPHOBLASTIC LEUKEMIA

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Some studies have suggested that osteoporosis might be one of the late consequences of childhood leukemia or of the treatment for this disease. However, little is known about the extent and the cause of a possible decrease in bone mineral density (BMD). In this study we have studied the BMD in 24 young adult survivors who were treated in our hospital because of childhood ALL. ***Methods***: 14 males and 10 females treated for ALL between 1972 and 1990 with different treatment protocols were included. All except 2, received radiotherapy (1800-3000 cGy) as part of their treatment. At the time of the investigation the mean age was 25.1 yrs (range 20.1-34.9) and the mean follow-up period was 14.1 yrs (range 5.3-21.7) after cessation of therapy. The BMD was measured with dual X-ray absorptiometry (DEXA; Hologic QDR-2000) in respectively the lumbar spine (LS), femoral neck (FN) and trochanter (FT) and at 1/3 and ultra distal (UD) in the radius. ***Results:*** These are expressed as Z-scores adjusted for sex and age, based on a reference database profided by the manufacturer.

Region	mean	range	95% CI
LS	-1.07	-4.15 to +0.47	-1.56 to -0.57
FN	-1.07	-3.60 to +0.98	-1.59 to -0.55
FT	-0.55	-2.77 to +2.11	-1.11 to +0.03
Radius 1/3	-1.76	-4.86 to +0.39	-2.28 to -1.24
Radius UD	-1.44	-4.46 to +0.38	-2.00 to -0.87

These data show that the BMD was significantly lower in survivors from ALL compared to the reference data. Some patients had very low BMD values; in 5 patients the Z-score for the LS and in 7 patients the Z-score at 1/3 in the Radius was > 2 SD below the mean normal value. The Z-score values per region for male and female patients did not differ significantly. ***Conclusions***: Young adult survivors of childhood ALL have a significantly lower BMD in comparison to age-matched controls; at least 20 % had one or more Z-score values 2 SD < mean. No sex difference was noticed. Studies are ongoing in our hospital to determine whether these changes in BMD occur during or after therapy and whether these changes are related to certain aspects of therapy, age and pubertal developmental stage and of course whether decreased BMD can be prevented.

P-21

BONE MINERAL DENSITY OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Skeletal abnormalities such as fractures and osteoporosis have been described in association with acute lymphoblastic leukemia (ALL). The objective of this study was to evaluate the bone mineral density (BMD) of children with ALL at diagnosis, during treatment and 1 year after cessation of treament. All children were treated according to protocol ALL-8 of the Dutch Childhood Leukemia Studygroup. Methods : BMD of lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (Lunar DPXL/PED). The results were compared to healthy age- and sex-matched Dutch controls and expressed as standard deviation scores (SDS). Twenty-two children (13 boys) participated in the study. BMD of 14 children was measured at diagnosis; 7 of them were also examined at 6 months. One child was measured at 6 months and three at 1 year during treatment and four 1 year after finishing the treatment. Results : BMD at diagnosis was not lower than controls. Lumbar spine BMD of the 11 children during treatment was significantly lower than controls ($p < 0.05$), a mean SDS of -0.96. The mean SDS of total body BMD during treatment was -0.88, not significantly below zero. The BMD of the children after treatment was not lower than controls. Conclusion : Lumbar spine BMD of children with ALL during treatment was significantly lower than controls. Either corticosteroids or cytostatic therapy may have a negative influence on BMD in these children, which may be reversible.

P-22

MODIFIED BFM TREATMENT IN 91 CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND MEDIASTINAL MASS IN MULTICENTER STUDY IN EAST GERMAN STATES

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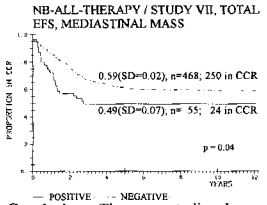
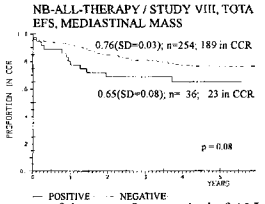
Approximately 12 % of all cases of ALL in childhood are classified as T-cell ALL. The presence of a mediastinal mass is a typical feature of this type of leukemia. There have been major advances in the past decade in the treatment of children T-lineage ALL.
Methods: Fifty-five (10,5 %) children with a mediastinal mass of a total of 524 children with ALL were entered into a multicentric controlled randomized study (ALL VII/81 corresponding to ALL-BFM 81) between September 1981 and December 1987. Patients were divided into three risk groups according to the BFM risk factor score (SR, MR, HR). For the duration of maintenance therapy, patients were randomized after 78 weeks to receive MTX and 6-MP for another 6 months or to receive a late intensification. From 1988 to 1991 291 children were treated according to a new modified BFM protocol (ALL VIII/87 corresponding to ALL-BFM 86). In 36 (12,4 %) patients were demonstrated thymic involvement. In this study MTX dosage was reduced from 5 g/m² (BFM) to 1 g/m². In both studies no mediastinal irradiation was administered.

Results:

		MEDIASTINAL MASS							
		ALL-VII/81				ALL-VIII/87			
		Positive		Negative		Positive		Negative	
		n	%	n	%	n	%	n	%
Patients, Total	55	100	468	100	36	100	254	100	
Complete Rem.		53	96	449	86	35	47	247	97
Relapses		24	44	149	32	9	25	40	16
Pat. in 1st Rem.		25	45	263	57	23	64	189	74
EFS (SD)		0.49 (0.07)		0.59 (0.02)		0.65 (0.08)		0.76 (0.03)	





Conclusions: These two studies show an improvement of the event-free-survival of ALL children with a mediastinal mass (49 % to 65 %) but a poorer prognosis than do those children without a mediastinal mass.

P-23

BONE TUMOR REVEALING PRE B LYMPHOBLASTIC PROLIFERATION: A RETROSPECTIVE STUDY OF 6 CASES

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Between 1991 and 1995, 6 patients admitted to orthopedic surgical department for bone tumor had a definite diagnosis of pre B lineage lymphoblastic proliferation and were treated in the Hematology-Oncology Unit.
Patients : 4 males, 2 females, mean age : 12 years (8-16). All presented with fever and localized bone pain, 4 of them had a tumefaction. Involved bones were : tibia (1), femur (1), radius and cubitus (2), humerus (1) and dorsal vertebra (1). None of the 6 patients ever had hepatomegaly, splenomegaly nor adenomegaly. For 1 boy a testis infiltration was secondarily noted. Mean duration of symptoms before diagnosis was 2 months (1 week-6 months). One patient with elbow tumefaction had a previous history of suspicion of tibial lymphoma 14 months before, unconfirmed by surgical biopsy and bone marrow aspirate.
Investigations results : peripheral blood cells counts were normal (5) or with presence of blastic cells (1). Standard bone X-rays showed osteolytic lesions (4) with subperiostal new bone formation (2) or normal images (2). All patients had increased uptake of technetium-99m : involved site alone (3), multiple sites abnormalities (3). Local MRI showed : low signal intensity on T1 weighted images and high signal intensity on T2 weighted images in 4/4 patients. 3/4 patients had contrast enhancement after gadolinium injection. Additional abnormal bone marrow signals were seen at other sites in 3 patients, one different child had multiple vertebral bone marrow abnormal signal. Histologic evaluation of bone specimen (4) and/or bone marrow biopsy (5) showed lymphoblastic proliferation. Bone marrow cytology confirmed homogenous (2) or heterogenous (4) lymphoblastic infiltration. Blast cells *immunophenotype by flow* cytometry were : CD19 and CD10+ (6), CD34+ (3), intracytoplastic Ig + (2), CD33+ (1), surface Ig - (6), CD3, 4,5,7,8 - (6). Cytogenetic : normal (2), hyperploïdy (1), 12 p- (1), failure (2).
Treatment : high risk B lymphoma chemotherapy followed by maintenance therapy (1); French ALL protocol FRALLE 93 (5). All patients achieved complete remission and are currently disease free (mean follow-up from CR : 12 months).
Conclusion : A bone tumor can reveal a pre B calla + lymphoblastic proliferation that can as well be considered as ALL or lymphoma with bone marrow metastases. This report underlines the interest of MRI in bone marrow imaging and flow cytometry immunophenotyping of blast cells to complete histological findings.

P-24

EFFECT OF rhGM-CSF ON IL-3 AND IL-7 SERUM LEVELS IN CHILDHOOD ALL.

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The aim of this study was to evaluate the influence of rhGM-CSF in vivo on Interleukin 3 (IL-3) and Interleukin 7 (IL-7) levels, in sera of patients with neutropenia and thrombocytopenia during chemotherapy. Forty-nine children (Group A) with Acute Lymphoblastic Leukemia (ALL) were subjected to a total of 102 chemotherapy cycles and were also treated with rhGM-CSF for 4-7 days depending on the degree of neutropenia and the chemotherapeutic agent. The control group (Group B) consisted of 22 children with ALL who did not present neutropenia but only thrombocytopenia during 85 chemotherapy cycles. Before, during and after the end of the treatment with rhGM-CSF and on a daily basis, white cell count, platelets, and hemoglobin levels in the blood and, also, IL-3, IL-7 and GM-CSF levels in the serum were determined. Results: Before administering the rhGM-CSF under conditions of neutropenia and thrombocytopenia, IL-3 was between 8.1-14.4 pg/ml and IL-7 between 97.5-115.8 pg/ml. The IL-3 levels were lower and IL-7 higher than those of the control group (IL-3 between 26.7-35.9 pg/ml, IL-7 between 18.3-31.4 pg/ml). The GM-CSF levels were between 2.7-4.4 pg/ml. They were lower than those of the control group (10.3-15.2 pg/ml). Two days after administering the rhGM-CSF, the IL-3 levels increased in proportion to the increase of leukocytes and platelets. The increase of IL-3, leukocytes, granulocytes and platelets varied between 7-22%, 2-25%, 4-18% and 1-22%, respectively. A steady increasing trend was observed during the following days up to 57-79% for IL-3. In contrast, IL-7 levels showed a decrease of 10-36% during the 2nd day of rhGM-CSF treatment reaching 38-52% at the end of the treatment. In the control group the IL-3, IL-7 and GM-CSF levels did not vary significantly. The number of platelet transfusions decreased from 5-65%. A detailed discussion, along with relevant statistical documentation, is given of the IL-3 and IL-7 levels in relation to the numbers of leukocytes, neutrophils, platelets and platelet transfusions. In conclusion, our results indicate that, rhGM-CSF not only increases the number of leukocytes and neutrophils restoring a normal immune response, especially in view of infections due to chemotherapy-induced neutropenia, but also induces an increase in the number of platelets, thus minimising the number of necessary platelet transfusions and preventing incidents of haemorrhage.

P-25

HYPOGLYCEMIA IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA DURING 6-MP AND MTX MAINTENANCE CHEMOTHERAPY

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Nausea,vomiting and anorexia are well known adverse effects of 6-mercaptopurine (6-MP) and methotrexate (MTX). We report on 9 children, aged 3 to 8 years, with acute lymphoblastic leukemia (ALL), on maintenance chemotherapy, including 6-MP and MTX, who developed symptomatic, ketotic hypoglycemia on fasting.
To study the blood glucose metabolism and fasting tolerance during maintenance chemotherapy for ALL we made a 16-hours-fasting-test on 15 children with ALL receiving oral daily 6-MP and weekly MTX. Nine of them (60%) generated manifest hypoglycemia after 12 to 16 hours of fasting (B-glucose 1.6 - 2.3 mmol/l). These 9 children with impaired fasting tolerance tended to have higher serum alanine aminotransferase (ALT) than the 6 children with normal fasting-test (mean 309 U/l, range 44-614, vs. mean 143, range 48-293). The median doses of 6-MP and MTX in these 9 children were 64.9 mg/m^2/d and 18.6 mg/m^2/wk, and in 6 other children 65.0 mg/m^2/d and 16.5 mg/m^2/wk, respectively. In two children the fasting-test was repeated after the termination of the maintenance chemotherapy and the impaired fasting tolerance was restored normal.
In conclusion 6-MP and MTX maintenance chemotherapy for ALL seems to impair postbrandial glucose homeostasis and involves a marked risk of hypoglycemia. This may also explain part of nausea and vomiting occuring in the mornings during 6-MP and MTX therapy.

P-26

TREATMENT RESULTS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL); A COMPARISON OF TWO BFM PROTOCOLS.

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This study aimed to assess treatment results in children with ALL and the prognostic value of risk factor (RF) as defined by Langermann et al.
It comprised two groups of children: 75 patients (29 girls and 46 boys) aged 14 - 172 mo. (median 60 mo.) treated with BFM 79 protocol and 32 children (14 girls and 18 boys) aged 12 - 149 mo. (median 71 mo.) treated with BFM 86 protocol. The median follow-up time was 63 and 28 mo. respectively.
The remission rate for both groups were similar (98.7% vs 98.9%; NS). The rate of death in remission was higher among children on BFM 86 protocol (2.7% vs 6.3%; NS). Children on BFM 86 protocol did slightly better, but the difference was not significant (p-EFS: 0.52 vs 0.69; NS, p-DFS: 0.54 vs 0.77; NS). When applied retrospectively for patients on BFM 79 protocol risk factor (RF) was much better in selecting children with good prognosis than the scoring system used in BFM 79 protocol. All children with RF < 0.8 and treated with BFM 79 protocol remain in Ist remission. Children with RF > = 0.8 seem to form a heterogeneous group in respect to prognosis.
The response to prednisolone was a strong independent prognostic factor for both studied groups.

P-27

PROGNOSTIC SIGNIFICANCE OF BLAST CELL KARYOTYPE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Karyotype abnormalities(KA) in acute leukemias are being the subject of study over the last 20 years. Many important prognostic features based on cytogenetic data have been discovered, mainly in adult patients (pts). The aim of our investigation was to determine the frequency and types of KA in our population of children (Ch) with ALL. Cytogenetic analysis was carried out in 37 nontreated pts with ALL (34 - nonB-All, 3- B-ALL). Different clonal KA were found in 15 pts (41%): all of them had nonB-ALL. Hyperdiploidy (N>50) was most common KA - 5/15 (33%). All these Ch were of "low risk" common-ALL and have now I complete remission (CR) >12 - 29 mos. One boy was hypodiploid with 43-45 chromosomes, he is in I CR (34+mos). Pseudodiploid karyotype was found in 9 pts (60%). Four pts had t(9;22). One of them relapsed in early CR, 3 others are in I CR after autoBMT (2+, 35+, 37+ mos). Five Ch had different KA including t(11;14), t(2;6), t(1;19), del 2 q-, del 11q-. Three of them are in I CR (>5-32 mos) and 2 have died in relapse. 19 Ch had a normal karyotype; 14 of them are in I CR and 5 pts relapsed. The EFS were 77%, 100% and 53% for Ch with nonB-ALL and normal, hyperdiploid and pseudodiploid karyotype respectively (group of B-ALL pts is too small for analysis). In conclusion, our data have confirmed the conventional opinion that common-ALL pts with hyperdiploidy have better EFS. Coincidence of this immunological type with such KA is universal prognostic feature.

P-28

ACUTE MEGAKARYOBLASTIC LEUKEMIA WITH OR WITHOUT DOWN SYNDROME IN CHILDREN

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The purpose of this study were comparison of clinical characteristics and prognosis of children with acute megakaryoblastic leukemia with or without Down syndrome(DS-AMKL or NDS-AMKL, respectively). Of 716 children with acute myelogeneous leukemia(AML), treated during the years 1986 to1994, 102(14.2%) cases were diagnosed as AMKL by use of platelet specific monoclonal antibodies and platelet peroxidase(PPO) reaction in electron microscopy. These AMKL included 41 cases(40%) of DS-AMKL and 61 cases(60%) of NDS-AMKL. Transient abnormal myelopoiesis(TAM) were excluded in this study.

Comparison of clinical characteristics showed no significant differences in age distribution, hemoglobin value, platelet count, leukocyte count, or percentage of blood or bone marrow blasts in children with DS-AMKL or NDS-AMKL. However, children with DS-AMKL were male predominant and had high incidence of hepatosplenomegaly. Cytogenetic abnormalities were variable, but abnormalities of chromosome 21 were detected on 80% of DS-AMKL and 20% of NDS-AMKL. All the cases of AMKL in children who recieved intensive chemotherapy, DS was no longer an independent risk factor. Event Free Survival(EFS) was almost the same in DS-AMKL and NDS-AMKL(54.4±8.5vs 49.2±7.1). On the other hand, serious toxicity requiring interruption of treatment and deaths resulting from toxicity occurred more frequent with DS-AMKL than NDS-AMKL. To prevent the toxicity related deaths, mild therapy should be considered to DS-AMKL.

P-29

GRANULOCYTIC SARCOMA IN CHILDREN WITH ACUTE MYELOBLASTIC LEUKAEMIA IN JOHANNESBURG, SOUTH AFRICA

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Granulocytic sarcoma, an extramedullary localized tumour of granulocytic progenitor cells, is reported to be present in 10 - 25% of children with Acute Myeloblastic Leukaemia (AML) at diagnosis in Africa. The purpose of this study was to determine the incidence of granulocytic sarcoma in patients with AML attending the Johannesburg and Baragwanath Hospitals, to determine whether there is an association with the presence of the 8;21 translocation [t(8;21)] and to report on the outcome of these patients.

A total of 88 patients with AML were diagnosed between 1985 and 1995 (69 Black and 19 White patients). Fifteen (17%) presented with granulocytic sarcoma at diagnosis, all of whom were black and male. The incidence of granulocytic sarcoma in the black group is 21,7% and 36% in black males.

The unusual presentations of granulocytic sarcoma often made the initial diagnosis problematic but by maintaining a high index of suspicion a definitive diagnosis was not unduly delayed.

Cytogenetic studies were performed on 13 patients with granulocytic sarcoma. The t(8;21) was seen in 9 patients (69%). All the patients with t(8;21) presented with orbital involvement.

Survival of the patients presenting with granulocytic sarcoma was considerably better than the overall survival in our patients with AML.

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CLASSIFICATION OF LEUKEMIC CELLS WITH ELECTRON MICROSOPY

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The introduction of electron microscopy in Hematology has contributed significantly to our knowledge of the ultrastructure and function of blood cells. In the present study, the ultrastructural characteristics of leukemic cells obtained from consecutive patients were classified in addition to the conventional morphologic, cytochemial and immunologic studies. First we found that certain cases of acute myelogeneouse leukemia (AML)- M2 showed smaller and fewer primary granules in the cytoplasm and, folding and indentation of nucleus, all of which were characteristic of monoblasts rather than myeloblasts. Secondly in acute megakaryoblastic leukemia (AMKL), blasts cells had characteristic findings of early erythroblastic leukemia, such as cytoplasmic theta granules and rhopheocytosis. Thirdly in acute lymphoblastic leukemia, which did not express CD13 and CD33, blast cells were negative for myeloperoxidase in light microscopy but positive in electron microscopy. Electron microscopy would provide some useful information to classify leukemic cells, especially in AML-M2 , AMKL and mixed-lineage leukemia, when used in tandem with conventional studies for biologic classification.

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RESULTS OF THE TREATMENT OF ANLL RELAPSES IN CHILDREN WITH INTENSIVE CHEMOTHERAPY INCLUDING MITOXANTRONE.

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Mitoxantrone (Mit) is an anticancer drug of antracendions group. It is reported as an effective in treatment of different cancers: breast cancer, NHL, refractory and relapsed ANLL. We used Mit (Novantrone, Lederle) for the treatment of 12 children with the first relapse of ANLL. All pts have received Mit in combination with high or intermediate doses of Ara-C (HD Ara-C) (HAM); Vepesid (VP 16) was added in the course in 5 pts (AME-H).

HAM: Ara-C 3 g/sq.m twice a day, days 1-4; Mit 10 mg/sq.m once a day, days 2-4.

AME-H: Ara C 1 g/sq.m twice a day, days 1-4; Mit 30 mg/sq.m once a day, day 2; VP-16 500 mg/sq.m once a day, day 4

The second remission was achieved in 9 pts (75%), in 3 pts leukemia was resistant. In 6 of 9 remitters the second relapse occured after 1-11 mo (median is 5 mo.), one pt died of intersticial pnevmonites 5 mths after allogeneic BMT, one pt died of septic shock in aplasia after 2-d course of chemotherapy. One pt with M2 subvariant with t(8;21)(q22;q22) is alive at 24 mo after autologous BMT following by beweekly IL-2 s.c. injections during the first 6 mo of 2CR. Probability of EFS during 24 mths is 8,3%.

Conclusion: Polychemotherapy with Mit was effective to induce CR in 75% of pts with 1-st ANLL relapse. Duration of the 2 CR was less than 6 mo in 75% of pts. ANLL relapse is virtually incurable even with intensive chemotherapy. It is necessary to develope principally new trends of treatment for those pts with ANLL relapse to whom allogeneic BMT is impossible.

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PERIPHERAL BLOOD STEM CELL MOBILIZATION FOLLOWING THE ADMINISTRATION OF 10 µg/kg G-CSF (Filgrastim) ALONE.

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Objective : This prospective study was designed to establish whether the administration of G-CSF alone could mobilize sufficient PBSCs to be collected and to plan transplantation in pts with advanced solid tumors.

Patients : 24 pts (neuroblastomas 16, medulloblastomas 2, lymphomas 3, various tumors 3) were mobilized in steady state hematopoïesis i.e. >21days after the last chemotherapy, in medullary CR prior mobilization and with no cytokines administration during the week preceeding the start of the priming.Median age : 5 yrs(1,5-17,5) and median weight:17 kg(10-47).

Results :G-CSF (10 µg/kg/day sc) was administrated once daily started 21 to 54days (median 26) after the last chemotherapy. Full blood count and reticulocyte subpopulation count as well as circulating CD34+cells, CFU-GM and BFU-GM levels were carried out daily.The timing of the collection was based on the daily evaluation of blood CD34+ cells kinetics data. Procedures were started 12 hrs after the 3thor 4th G-CSF dose, using Cobe Spectra. Baseline median number of WBC was 2,8.10^9/L(1,2-10,6), platelets 227.10^9/L(50-525), HFR 2,6.10^9/L(0,6-7,9), CD34+ cells 2.10^6/L(1-13). At the 1st day of collection, the median number of WBC was 34,8.10^9/L(7,8-64,7), CD34+ cells 20.10 ^{6}/L(1-112). Median CD34+ cells number/ kg / blood volume processed obtained after 3th,4th and 5th G-CSF dose were respectively:1,25.10^6/L (0,8-1,7), 1,1.10^6/L (0,1- 4,9), 1,1.10^6/L (0,3- 6,8). There was a close correlation between the number of circulating CD34+cells and the number of progenitor cells in the leukapheresis products.□

Conclusion : As the progenitor cell release induced by G-CSF has not been extensively evaluated in children the optimal timing for collection was based on the close monitoring of the mobilization kinetics.The maximum number of progenitor cells can be collected between day 3 and day 6 of G-CSF administration and peak blood values of circulating progenitor cells were occured after median 4th dose of G-CSF. We found a strong correlation between HFR and CD34+ cells levels:HFR,easy to evaluated , is an early indicator of the circulating progenitor increase during mobilization.There were no complications related to mobilization and. hematopoietic recovery post graft was successful.

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PREVENTION OF SIDE EFFECTS OF THERAPY BASED ON THERAPEUTIC DRUG MONITORING ON CHILDREN AFTER BONE MARROW TRANSPLANTATION

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One of the most effective method of prevention drugs toxicity after bone marrow transplantation (BMT) is adjustment of dosage regimen of the drugs using drug level monitoring (DLM) data. DLM of Cyclosporin (CSA) and Methotrexate (MTX),Vancomycin (V) and Amikacin (A), Phenitoine (P) were carried out on 12 children after allo-and auto- BMT. Prevention of nephrotoxicity due to simultaneous administration of number of such nephrotoxic drug as CSA, V and A together with Amphothericin is particularly important. Based on our data we conclude that correction of V and A doses according to clearance of creatinin (Cr) isn't valuable, and only DLM allowed normalize kidney function without interrupting V, A and CSA administration. Max.concentrations of A in samples taking in 1 h after administration of A 10,0-14,8 mg/kg/d (av.13,6) as a single one-hour infusion were within interval 12,2-43,1 mcg/ml. Peak level of A - 34,9 mkg/ml was confidently reason of increasing value of Cr and urea above normal range in 1 patient from 8. 50% of patients required to decrease doses of A by average 26% according to therapeutic range. Average dose of V were 37.6 mg/kg/d (28,6 - 45,5 mg/kg/d). 7 infusion of V accompanied by nephrotoxity. In all of this cases max. concentrations of V (sampling in 1 h after infusion) were higher then 37 mkg/ml (up to 65 mkg/ml). 56% of patients needed decreasing doses of V, 33% - increasing and only for 11% of patients initial dosage regimen was acceptable for therapy. All patients received CSA needed to adjust doses from initial calculated dose. Normal kidney's function allowed avoid interrupting CSA administration and carry out effective prophylactic of GVHD. There weren't any cases of acute GVHD III-IV st. and only one patient had chronic GVHD. Concentrations of P after administration in regular scheme without corrections were lower then min. effective concentration due to the metabolic induction on 8th day. Using DLM of P gave possibility to decrease neurotoxicity of conditioning regimen and post-transplantation therapy and only one of our patients had episode of seizures. Furthermore no patient of 12 under DLM of V hadn't symptoms of veno-occlusive disease. Due to DLM individual dosage regimens were developed that allowed the prevention and treatment the main complication of BMT - GVHD, serious infections and toxicity of chemotherapy more effective.

P-34

EX VIVO EXPANSION OF CORD BLOOD-DERIVED PROGENITOR CELLS. POTENTIAL UTILITY AND POTENTIAL LIMITATIONS IN TRANSPLANTATION.

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Cord blood seems to represent an easily accessible source of transplantable stem cells capable to reconstitute hemopoiesis after transplantation. We studied the phenotypic characteristics of umbilical cord blood (UCB) mononuclear cells and the influence of combination of growth factors on proliferation and differentiation of the hemopoietic progenitors in vitro. Samples (n=25) were taken from healthy neonates at the 38-40 weeks of gestation. Phenotypic analyses were performed by one and two colour flow cytometry using a Coulter Epics flow cytometer. Colony forming cells with mixed lineages potential (CFU-GEMM), primitive erythroid progenitors (BFU-E) and progenitors of the granulocyte and macrophage lineages (CFU-GM) were quantified by methylcellulose culture. We observed that the expression of the surface antigens of the T-lineage was higher than that of the B-lineage. The HLA-DR antigen was rather minimally expressed while UCB mononuclear cells contained a mean of 2,29±1,8 CD34$^+$ cells. The effect of different cytokines was as follows: The combination of Epo, IL-3, GM-CSF exibited the strongest ability to promote CFU-GEMM. Significant CFU-GM formation resulted following addition of GM-CSF, IL-3 nad G-CSF in a dose dependent way. Fetal BFU-E showed on optimal growth in the presence of Epo alone, suggesting the presence of an additional subpopulation of BFU-E in fetal blood with unusual growth characteristics. These results suggest that cord blood is a rich source of immature hematopoietic progenitor cells when it is compared with bone marrow progenitors.

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Myelodysplastic syndrome in 13 children treated with high dose chemotherapy and bone marrow transplantation.

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Between 1987 and 1995, 13 children with MDS were treated : the purpose was to induce complete remission for those in transformation and then to perform BMT. 11 children have been grafted : 4 from genoidentical donor, 1 from related mismatched donor, and 6 from matched unrelated donor. Conditioning regimen was the same for all patients : F TBI, ARA-C (24g : 4 cases - 18g : 7 cases) and melphalan.

Our group consisted of 5 boys and 8 girls with a median age of 7y.6m (1-14 y.). According to FAB classification 6 cases were CMML, 6 were RAEB-T and 1 was in leukemic transformation (ANLL). Cytogenetics was available for all but one patients : 3 monosomy 7, 1 trisomy 8, 1 trisomy X, 1 del 12q, 1 complex caryotype (including monosomy 7), and 5 normal caryotypes.

One child with CMML without any blast cells in bone marrow did not receive any chemotherapy before BMT was performed from a genoidentical donor (alive, 43m follow up). The other twelve received chemotherapy :

* 1 received ARA-C small dose and VP16 but died from aspergillosis before BMT. Another one died from aspergillosis after HD ARA-C (36g/m²) and mitoxantrone (30mg/m²). One received a 1st BMT after conditioning regimen with VP16 - Misulban, Cyclophosphamide, and relapsed soon after ; a second CR was achieved after HD ARA-C (24g/m²) and a 2nd genoidentical BMT was performed (alive - 7y 6m follow up).
* The last nine received HD-ARA-C + anthracyclin (mitoxantrone 30mg/m² = 8 ; amsacrine 30mg/m² = 1) and hematological remission was achieved in 6 cases. One of them relapsed while waiting for a phenoidentical donor ; BMT was performed later, after a 2nd course of chemotherapy and the patient died from severe toxidermia. Among the 5 patients grafted soon after hematological remission 3 are alive (34m, 25m, 22m follow up), 1 relapsed 1 y. after BMT and 1 died of disseminated aspergillosis. Three patients were not in remission at BMT : 2 died from toxicity (1 acute GVHD 1 pneumopathy) and 1 is alive (17m follow up)

Combined HD ARA-C and anthracyclin chemotherapy seems to be effective in childhood MDS in transformation : 7/10 went into remission (1 relapsed). Nevertheless BMT should be performed as soon as possible before hematological and clinical progression. Six patients are alive, all after BMT and 7 died (2 before BMT, 4 from BMT related toxicity and 1 from relapse).

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL OR BONE MARROW TRANSPLANTATION IN CHILDREN WITH AGGRESSIVE NON-HODGKIN'S LYMPHOMA (NHL) IN FIRST COMPLETE REMISSION (CR)

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High-dose chemotherapy followed by autologous transplantation (auto-transplant) has been accepted as effective therapy for patients with NHL. Several clinical trials have demonstrated that auto-transplant can be curative in patients with aggressive NHL and that patients are more likely to be used if treated early in the course of their disease at a time when their tumors remain sensitive to chemotherapy. Auto-transplant should be carried out before the NHL become resistant to chemotherapy. We hereby treated 9 children with intermediate-grade and high-grade NHL (the Working Formulation) in first CR with auto-transplant. There were 5 males and 4 females from 3 to 16 years of age, and stage at diagnosis included 5 of stage III and 4 of stage IV. All patients received combination chemotherapy according to NHL890 protocol by the Children's Cancer and Leukemia Study Group of Japan, and achieved CR. Five received a preparatory regimen consisting of MCNU (450mg/m²), etoposide (1600mg/m²), cytarabine (16g/m²) and CY (100mg/kg), and 4 received busulfan (16mg/kg), etoposide (1000mg/m²) and CY (100mg/kg). Six received peripheral blood stem cells and 3 received unpurged bone marrow grafts. All patients are alive and well in unmaintained CR with a median follow up period of 24.3 months (range, 3 to 51 months) after transplantation. Although the role of high-dose chemotherapy followed by auto-transplant as the part of initial treatment should be investigated by a randomized study, our data suggest the safety and efficacy of auto-transplant in patients with aggressive NHL in first CR.

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BONE MARROW TRANSPLANTATION (BMT) FOR REFRACTORY LANGERHANS CELL HISTIOCYTOSIS (LCH).

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Although patients with localized, single system LCH may have an excellent outcome with minimal or no treatment, a significant proportion of children with disseminated disease may undergo a progressive disease with fatal outcome despite chemotherapy and or immunotherapy. Since LCH derives from hyperactivation and proliferation of macrophage progeny cells that originate in the bone marrow, there is a rationale for use of BMT for treatment of advanced, resistant LCH. Only six cases of LCH treated with BMT have been reported in the literature, including two cases of autologous BMT. Of them only one was less than 14 years of age and all were conditioned also with Total Body Irradiation (TBI).

We report a 4-year-old male, with biopsy proven, disseminated LCH with skin rash, lymphadenomegaly, massive hepato-splenomegaly, anemia (6.4 gr/dl) and thrombocytopenia (29 x 10^9/L). LCH was not controlled by front-line monotherapy with etoposide, neither by rescue treatment with combined chemotherapy (vinblastine and etoposide) and immunotherapy (steroids and cyclosporin). On month 11 after the diagnosis, he underwent allogeneic BMT from the HLA-matched, 7-year old sister, heterozygous for β-thalassemia. The conditioning regimen consisted of busulfan (3.5 mg/kg for 4 days), cyclophosphamide (60 mg/kg for 2 days), melphalan (140 mg/m²). He received 6.4x10^8 mononucleated bone marrow cells/kg b.w. Cyclosporin (1 mg/kg/day c.i. i.v. days -1 to 24, then 12 mg/kg/day orally days 25-140) was given as prophylaxis against graft-versus-host disease. After successful engraftment, clinical manifestations of LCH disappeared and the patient is asymptomatic and disease free 22+ months after the transplantation.

Preliminary experience suggests that BMT has a potential for cure of refractory LCH even in very young patients. This experience suggests that similarly to observations in other histiocytoses, TBI might be omitted from the conditioning regimen.

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AUTOTRANSPLANTATION WITH PERIPHERAL BLOOD PROGENITOR CELLS IN CHILDREN: INFLUENCE OF CD34 DOSE IN ENGRAFTMENT KINETICS

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Introduction: PBPC have been accepted as a stem cell source to support high dose chemotherapy in children with cancer. There are few and controversial data concerning engraftment kinetics in pediatric patients. We analyse factors affecting engraftment in pediatric patients mobilized by filgrastim alone.

Patients and methods: 40 children with different malignancies (5 ALL, 6 ANLL, 6 NHL, 3 HD, 8 RBM, 5 NRB, 5 CNS, 1 EWS and 1 WT) were autografted with PBPC between Nov 93 and Dec 95. There were 25 males and 15 females. The mean age and weight were 8 yr.(1-16) and 30 kg (9-60) respectively. All patients received G-CSF 12 microg/kg/day sc for 4 days and collections were started on 5th day using a Cobe-Spectra cell separator through a central venous catheter. In patients weighing < 25 kg the extracorporeal line was primed with a unit of packed red blood cells. The patients received myeloablative regimens in accordance with their malignancies and on day 0, the PBSC were thawed and infused. G-CSF was given from day +1 until hematologic recovery.

Results: A total of 54 apheresis were performed (mean 1,35 aph/pt). The mean of infused cells were 9.9 x10e8/kg MNC; 12.6x10e4/kg CFU-GM and 7.21x10e6/kg CD34+ cells. All patients except one who died on day 5 after infusion showed rapid engraftment. Patients required a mean of 9.5 days to achieve granulocyte recovery (Neutrophils >0.5x10e9/L) and 18.1 days for platelet recovery. In a multivariate analysis we found that the number of CD34+ cells infused was the most important factor correlating with neutrophil and platelet recovery. We found a good correlation between infused CFU-GM and neutrophil recovery but not between this factor and platelet recovery. We have observed a threshold effect for the CD34+ cells. Patients receiving > 5x10e6 CD34+ cells/kg achieved a significantly more rapid granulocyte and platelet engraftment than did patients receiving < 5x 10e6/kg CD34+ cells.

Conclusion: The number of CD34 cells infused is the most important factor influencing time to hematological recovery.e

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PERIPHERAL BLOOD PROGENITOR CELL COLLECTION AND TRANSPLANTATION IN CHILDREN WITH CANCER.

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Peripheral blood progenitor cells (PBPC) mobilized by hematopoietic growth factors are increasingly being used for autologous hematopoietic support following high-dose chemotherapy. However, little clinical information is available on the feasibility of using this method for treatment of cancer in children.

PBPC were collected in 14 children from January 1995 to January 1996. The diagnoses were rhabdomyosarcoma (n=3), PNET (n=3), NHL (n=2), osteosarcoma (n=2), malignant teratoma (n=2), Wilms tumor (n=1) and neuroblastoma (n=1). The mean age was 9.7 (1.5-16) years and the mean weight 37 (10-72) kg. The interval from initial diagnosis to PBPC was 6.3 (2-19) months. All patients received lenograstim (G-CSF; 5 μg/kg) after various chemotherapy regimens for mobilization of PBPC. The collection of PBPC (with Cobe Spectra Apheresis System) was started when peripheral blood CD 34+ cells were mean 76 (1.54-240) x 10^6/L.

A total of 24 leukaphereses were performed (mean 1.7 aphereses/patient). The total duration of PBPC collection was 222 (80-308) minutes/patient. The total amount of CD 34+ cells collected was 5.35 (1.5-12.2) x 10^6/kg.

Six patients have been transplanted. The mean days of ANC > 0.5 x 10^9/L and platelet > 30 x 10^9/L were 10 (7-20) and 19 (12-38) days, respectively. The mean duration of hospitalization was 18 (15-24) days.

PBPC collection and high-dose chemotherapy with subsequent rescue with PBPC is a safe procedure even in small children with cancer.

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AUTOGRAFTING WITH PERIPHERAL BLOOD STEM CELLS IN CHILDREN WITH SOLID TUMORS.

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Candidates for high-dose chemotherapy or bone marrow transplantation whose marrow is either fibrotic or infiltrated with malignant cells may circumvent this obstacle by employing peripheral blood stem cells (PBSC) for autologous hemopoietic rescue.

Since May 1989 we have performed leukaphereses in 54 pts (36 males/18 females) with solid tumors by means of a Baxter CS-3000 Plus continous-flow cell separator. Patient age ranged from 13 months to 18 year (median 8 years), weight ranged from 10 to 71 Kg (median 24.5 Kg). 216 PBSC collections were performed, with an average of 4 collections per patient (range 1-9). The PBSC were then frozen and stored at -196°.

24 patients were subsequently given the thawed product. 2 patients performed autografting with both BM and PBSC after myeloablative therapy and had un uneventful recovery. 22 patients were grafted with PBSC only after myeoloablative therapy. The myeloablative regimen for all patients consisted in Cyclophosphamide 60 mg/Kg days 1 and 2, Thiotepa 250 mg/m^2 days 1,2 and 3, Etoposide 250 mg/m^2 days 1,2 and 3.. Median recovery time (defined as ≥500 PMNs and ≥ 50.000 PLTs/mm^3) was 16.5 days (range 12-22 days) for PMNs and 19 days (range 13-25 days) for PLTs. 5 patients were given PBSC support after submyeloablative therapy; median time was 6.5 days (range 5-13 days) for PMNs and 10 days (range 8-15 days) for PLTs. The introduction of CSF allowed for a significantly shorter recovery time for recovery PMNs. Both G-CSF and GM-CSF were given at 10mcg/kg s.c. (PMN >1,000 ^{mm3} and PLTs >50.000/ ^{mm3} was obtained after 12.5 days (range 5-15) and 19 days (13-25) rispectively.

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USE OF PERIPHERAL BLOOD STEM CELLS INSTEAD OF BONE MARROW FOR ALLOGENEIC TRANSPLANTATION IN CHILDREN

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Introduction: PBSCs mobilized by G-CSF provide a rapid and sustained hematopoietic reconstitution after autologous transplantation, facilitate earlier discharge and avoid risk of general anesthesia and discomfort of bone marrow harvesting. Transplantation of allogeneic PBSC may have similar advantages. Larger randomised studies are now required but preliminary experience therefor suggests that the severity of acute GVHD is not increased despite the higher number of T-cells in stem cell harvests. Here we describe our experience in four children with hematologic malignancies.

Patients and methods: Donor and recipient's characteristics are shown in table 1.

Diagnosis	Age D/R	Sex D/R	Weight D/R
JCMML	17/1	F/F	60/10
ALL 2CR	18/8	F/M	85/40
ALL 2CR	23/10	M/M	75/40
ALL 2CR	1/8	M/M	10/42

The donors were mobilized by G-CSF 10 microg/kg/day s.c for 4 days. On 5th-6th day a large-volume leukapheresis were performed using a Cobe-Spectra cell separator using peripheral vein access in three patients and a femoral catheter in the other one. Three patients required only one apheresis and the other patient required two.

Results: The median number of infused cell per kg recipient's weight were: 14.85 x10e8/kg MNC, 12..22 x10e6/kg CD34+ cells and 2.17 x10e8/kg CD3+ cell.s. The patients received BU-CY-VP16 (JCMML) or TBI-CY (ALL) as conditioning regimens. The GVHD prophilaxis was CsA/short course Mtx in all cases. The hematologic recovery times are shown in table 2.

ANC< 100	ANC>500	ANC>1000	Plat.>20000	Plat.>50000
9	16	17	16	20
6	12	12	12	18
5	14	14	12	15
7	16	18	16	20

Two patients developed acute GVHD that responded to steroid treatment while the other two pt.did not developed acute GVHD. Only one patient developed chronic GVHD at the moment of this report and he died 5 months later of fungal infection. The other three patients are alive and in complete remission.

Conclusion: These cases suggest that allogeneic PBSC transplantation in children achieves rapid hematopoietic recovery without severe GVHD.

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HIGH DOSE THERAPY AND AUTOLOGOUS HEMATOPOIETIC PROGENITOR TRANSPLANTATION IN EWING'S TUMORS IN COMPLETE REMISSION

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Patients and methods:

We retrospectively studied 24 patients with Ewing's tumors in first or second complete remission who received megatherapy and bone marrow or peripheral blood rescue (PBPC) at Spanish Centers.

There were 15 males and 9 females with a median age of 9.92 yr (range 2-15). All had high risk Ewing's sarcomas: 9 patients had metastatic disease at initial diagnosis, 8 had local or metastatic relapse and 8 patients had tumoral volume > 100 mL. Primary treatments included conventional chemotherapy in all patients. Local treatment of the primary tumor included surgery (8 pt.) and radiotherapy (20 pt.). At transplantation time 16 patients were in 1CR (83.3%), and 8 pt. in 2CR (33.3%). Bone marrow harvesting were used in 10 children and 14 patients received PBPC.

Results:

At the time of this report, 9 of the 16 patients (56.25%) and 2 of the 8 patients (25%) who underwent transplantaton in first and second remission respectively are alive and in complete remission, with a median of follow up since high dose chemotherapy of 26.64 months (range 1-118 months). Two patients (18.18%) died of procedure-related complications.

The actuarial survival after megatherapy showed a global EFS of 34.43% at 118 months. Patients transplanted in 1CR showed an EFS of 48.68% at 118 months, and patients autotransplanted in 2CR had an EFS of 0% at 20 months.

Conclusion:

We conclude that patients with high risk Ewing's sarcoma may benefit from consolidation treatment with megatherapy in first remission. Poor results are found in second remission.

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PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION (PBPCT) IN CHILDREN WITH POOR PROGNOSIS OR RECURRENT SOLID TUMOURS

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Peripheral Blood Progenitor Cell Transplantation (PBPCT) is increasingly being utilized to treat childhood cancer. While the feasibility of PBPCT in adults is well-established, experience with this methodology in children is still limited. We review our experience with 17 children of mean age 11.2 years (range 3.0-16.7) who underwent PBPCT for recurrent solid tumor disease (non-Hodgkin lymphoma [1], Hodgkin lymphoma [5], neuroblastoma [2], Ewing sarcoma [2] and Wilms tumor [1]) and for poor prognosis solid tumors (Ewing sarcoma [2], ovarian PNET [1], rhabdomyosarcoma [1] and neuroblastoma [2]), using disease-specific myeloablative regimens. Four of these children received autologous bone marrow and PBPC infusion; the remaining 13 received PBPC re-infusion alone. The mean patient weight was 39.9 kg (range 12.0 - 63.0 kg). A mean of 8.2×10^8 (95%CI 6.3, 10.9) MNC/kg body weight was re-infused to each patient. In vitro-assay of Granulocyte-Monocyte Colony Forming units (CFU-GM) showed a mean of 77.2×10^4 CFU-GM/kg (96% CI 0.7, 279.7) for each re-infusion. Flow cytometric analysis of each re-infusion demonstrated a mean of 13.6×10^6 (95%CI 1.7, 46.3) CD34+ cells/kg. Trilineage engraftment occurred in all except for 2 patients. One died of infection five days post PBPCT and the other of multiorgan failure forty-one days post PBPCT showing only partial engraftment. The mean number of days to ANC $>0.5 \times 10^9$/L was 11.4 days (95% CI 8.0, 21.0), to ANC >1.0 was 12.6 days (95% CI 9.0, 21.0), and to unsupported platelet recovery> 20×10^9/L was 14.9 days (95% CI 9.0, 28.0). The mean number of days to platelet recovery $>50 \times 10^9$/L was 21.0 days (95% CI 11.0, 63.0) and for >100 was 34.2 days (95% CI 14.0, 92.0). These preliminary data demonstrate the feasibility of PBPCT in children. Our results also demonstrate rapid platelet and granulocyte engraftment with PBPCT.

P-44

CONVENTIONAL THERAPY VERSUS MEGATHERAPY FOLLOWED BY HEMATOPOIETIC STEM CELL RESCUE IN THE TREATMENT OF UNRESECTABLE EWING'S SARCOMA

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Since 1979 to 1994, 21 patients (pts) with localized inoperable Ewing's Sarcoma -10 males and 11 females, aver.age 12y, were treated by conventional chemotherapy. Primary tumor was localized in pelvic region (19 pts), tibia and fibula (1 pts) and cervical spine (1 pts). Radical surgery could not be performed due to localization of the tumor or parents refusal (tibia and fibula). All pts received chemotherapy (protocol T-11 or T-2 {16 pts}, cisplatin + adriamycine {1 pt} and CESS protocol {4 pts}) and radiotherapy. Fourteen children were operated by partial resection of the tumor. Eighteen pts relapsed in aver. period of 15.5m (1-84m), 16 children died from disease progression and 1 for sepsis (aver.period to death 17.1m). 1 child is still alive with sign of disease. Only 3 children are DFS for 10.6y (8y-16y).

Since 1992 all children with inoperable Ewing's sarcoma have been indicated to conventional induction chemotherapy followed by megatherapy and autologous hematopoietic stem cell rescue (AHSCT-both BM and PBSC). The conditioning regimens were: carboplatin + VP-16, carboplatin +VP-16 and melphalan with or without TBI (12 Gy). Up to December 1994 six children (aver.age 11.8y; 4 boys and 2 girls) underwent this procedure (4 pts disease in pelvic area, 1 pt in humerus, 1 pt in femur). All pts recovered and are alive (NED). Median post-transplant follow up is 18.8m.

Preliminary results look promissing and maybe indicate superiority of megatherapy followed by AHSC rescue compared to conventional therapy in children with unresectable tumors.

P-45

EFFECTS OF MEGATHERAPY ON EXTRAOSSEOUS TUMORS OF EWING'S SARCOMA FAMILY IN CHILDREN AND YOUNG ADULTS.

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The effects of myeloablative high-dose chemotherapy rescued by ABMT or PBSCT (megatherapy) on high-risk subsets of solid tumors other than neuroblastoma are still debated because of limited experiences. The purpose of this paper is to evaluate the effects of megatherapy in patients with extraosseous tumors of Ewing's sarcoma (ES) family in regard to the important prognostic factors in comparison with those who underwent conventional therapy.

Since 1984, ten patients with extraosseous ES/PPNET were treated in Keio University Hospital. The ages ranged from 7 months to 19 years with a median of 12 years. Six patients had primary tumors around thoracic or lumbar vertebras invading spinal canal as dumbbell type. Three patients had pelvic mass spreading into the retroperitoneum, and one had a mass in the buttock. Of these, 6 patients received megatherapy after complete response (CR) to induction therapy consisted of conventional chemotherapy and radical surgery +/- irradiation. Four patients were treated with conventional chemotherapy and palliative surgery +/- irradiation. Conventional chemotherapy regimen included CPA, ADM, VCR, ACD, CDDP and VP-16. Conditioning regimen for megatherapy consisted of L-PAM (or IFO), CBDCA and VP-16. Ablated marrow was rescued by ABMT in two patients, by PBSCT in two and by ABMT+PBSCT in two.

Among the patients who received the megatherapy, tumor relapsed with lung metastasis in two patients who had had lung metastases before achieving CR, and they died of disease 16 months after their megatherapy . Remaining four patients, who had had no evidence of hematogenous metastasis, remain disease free 4 to 41 months (mean, 19.5 months). Continuous oral administration of 5-fluorouracil has been done as post-transplant therapy in recent two patients. All four patients who were treated only with conventional therapy died of disease within 10 months (mean, 4.5 months).

The megatherapy could improve the prognosis of high-risk extraosseous tumors of ES family. Occurrence of relapse in the patients who have hematogenous spreading before megatherapy suggests a considerable contamination of collected progenitor cells by tumor cells. 5-fluorouracil may be useful for post-megatherapy treatment.

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SERUM ADHESION MOLECULES, ICAM-I (CD_{54}) AND CD_{44} LEVELS AND THEIR TISSUE EXPRESSIONS IN PEDIATRIC HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMA

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Levels of soluble intercellular adhesion molecules ICAM-I (CD_{54}) and CD_{44} were measured in serum samples obtained from 37 children with M. Lymphoma (ML) at diagnosis and remission with a sandwich enzyme immuno-assay (ELISA) method. Sixteen cases with Hodgkin's disease (HD), 13 non Hodgkin's lymphoma (NHL) and 8 Burkitt's lymphoma (BL) were included in the study. There were 22 males and 15 females ranging in age from 3 to 15 years. Twelve age matched children were used as controls. Expression of adhesion molecules in tumor tissue samples was assessed by APAAP technique. Median levels of serum ICAM-I were significantly higher in HD than in controls and other ML cases. However, no correlation was found between disease stage, histology, B symptoms and ICAM-I levels in HD. Serum CD_{44} (sCD_{44}) was also elevated in all patients with ML, highest being in HD before treatment. sCD_{44} levels correlated with the stage of the disease, B symptoms and increased ESR in HD. Tissue ICAM-I (CD_{54}) and CD_{44} expressions were also found to be increased in advance stage of the diseases. Serum CD_{44} and ICAM-I levels were decreased significantly in patients with complete remission. These preliminary results suggest that determinations of serum ICAM-I and sCD_{44} may be useful in evaluation and/or monitoring of treatment response in patients with ML.

P-47

TREATMENT OF HODGKIN'S DISEASE (HD) IN CHILDREN WITH MOPP/ABVD WITHOUT RADIOTHERAPY

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OBJECTIVES
Treatment of children with HD with non-cross resistant drugs, without radiotherapy.

BACKGROUND
Since the introduction of MOPP, children with HD can be treated with chemotherapy alone. Side-effects of irradiation will be absent, but from alkylating agents in MOPP gonadal dysfunction and secondary malignancies are known. Combination with non-cross-resistant drugs in ABVD might give better results and might decrease occurrence of side-effects.

DESIGN/METHODS
From 1988 to 1993 children with HD were treated with alternating MOPP and ABVD (3x MOPP, 3x ABVD).

RESULTS
21 children (7 F/13 M), age 5 to 18 years (median 14 years) were included. Clinical stages: I 7 pat, II 8 pat, III 5 pat, IV 1 pat. Pathology: 2 LP, 17 NS, 1 MC. In 1 patient only cytology was done. Two children relapsed. Toxicity: no decrease on cardiac ultrasound, no pulmonary effects by CO-diffusion, 6/10 had normal FSH and LH values, no thyroid dysfunction, no secondary tumors.

CONCLUSION
Children with HD can be treated without radiotherapy. Gonadal dysfunction seems less frequent than with MOPP. Prolonged follow-up is needed.

P-48

LONGITUDINAL FOLLOW-UP OF T-CELL SUBSETS IN CHILDREN WITH HODGKIN'S DISEASE

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Hodgkin's disease (HD) pts have an impaired mediated immunity with defective T-cell function and a reduced number of circulating T-cells, as well as alterations in the relative proportions of T-cells subsets. These abnormalities may present for years after successful treatment. However, there is no general agreement regarding the T-cell profiles in HD during remission.

Patients and Methods: Between 1979 and 1991, 30 pts, aged 4-16 years with HD were treated according to stage with 4-6 alternative cycles MOPP/ABVD +IF RT. regarding to pts distribution according to histology, 7 cases were HD lymphocyte prevalence (LP), 14 HD nodular sclerosis (NS), 9 HD mixed cellularity (MC). Eleven pts were stage I HD, 9 pts stage II HD, 9 pts stage III HD and 1 patient stage IV. B disease was present in 8/30 (27%) pts. All pts achieved complete remission. Absolute numbers and mean percentages of T-cell subsets was incestigated by their reactivity to MoAb (OKT3, OKT4, OKT8, OKIa, Ortho, NJ). The median follow-up time is 48 months. All results were compared with 59 well sex- and age-matched controls.

Results: The mean absolute lymphocyte counts as evaluated in MCG stained whole blood smears were within normal values. The pts had reduced mean percentage of total T-cell ($P<0.005$). The percentages and numbers of the pts OKT4+ cells were decreased ($P<0.05$), while their OKT8+ cells within normal values. OKT4+/OKT8+ cell ratio were also decreased ($P<0.05$).

Conclusion: The findings indicate a helper-cell depletion in HD during remission. Presence of immunoabnormalities after successful treatment suggests that they may be host-related and may preceede development of the disease.

P-49

INVOLVEMENT OF THE BONES IN CHILDREN WITH HODGKIN'S DISEASE

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We had treated 367 children and adolescents with Hodgkin's disease (HD) in 1982-1995. 9 of them (2,5 %) had specific bone lesions. All cases were detected by X-ray as irregular bone destructions, in 4 patients those data were confirmed by osteoscintigraphy, in 2 - by CT. In 2 bone lesions had large soft-tissue component detected by sonography as a mass with medium, uneven echogenety.

5 of those patients had initial involvement of the bone (pelvis - in 3, humerus - 1, femur - 1, sternum - 1). They underwent chemotherapy of various combinations and irradiation including the affected bones, TD to which were 40-45 Gy. 2 of them died of progression of HD, 3 are alive 1-4 years after initial treatment without bone relapse.

4 other children had bone lesions as a site of relapse (humerus - 1, scull- 1, pelvis - 2). 1 of them died of progression of the disease, 2 are alive undergoing anti-relapse treatment, 1 was alive 1,5 years after anti-relapse treatment (later lost for follow-up).

Active treatment can bring durable remissions or at least good pallitive effect in children with HD bone lesions.

P-50

CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) IN CHILDREN: FIRST REPORT FOR RUSSIAN CHILDHOOD MDS STUDY GROUP

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CMML is the most controversal entity among childhood hematological malignancies. Some large series have been recently reported, however, there is no final agreement on the definition of this disorder. There are also such diagnosis as JCML and infantile monosomy 7 syndrome and relationships between them and CMML have not been determined clear. In this regards we have evaluated our group of 23 children with CMML with the aim to analyse the most characteristic features of this disorder. The age of patients (pts) ranged from 3 months (mos) to 14 years (Me 3 years) and M/F ratio was 1.9. The main hematological features included: anemia/ /thrombocytopenia (83% of cases), increased leucocyte count (52% of cases), blast cells in peripheral blood (87% of cases) and absolute monocytosis (mean $4.5\pm0.2^{9}/l$) in all pts. In 43% of cases BM blast cells count was normal and exceeded 5% in 57% of cases. The two/three cell line dishemopoiesis was revealed revealed in all cases.Treatment approaches (1st line) included monotherapy with 6-mercaptopurine/busulfan (13 pts), low-dose AraC (5 pts), AML-like chemotherapy (5 pts). The survival of CMML pts ranged from 1 to 102+ mos (Me 16 mos), 13/23 pts (57%) transformed into AML. Five children (22%) are alive in partial remission during 30-100 mos (Me 77 mos). In conclusion, our data confirmed the fact of heterogeneity of childhood CMML. The further data collection, search of prognostic factors and elaboration of best treatment approaches are warranted.

P-51

RAS MUTATIONS IN CHILDREN WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

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Chronic myelomonocytic leukemia (CMML) in childhood is a rare hematopoietic disorder clinically distinct from adult-type CMML. Leukemic cells of adults with CMML have been reported to bear mutations in the *ras* oncogenes in more than 50% (reviewed in Int. J. Hematol., 57:99, 1993). We seek to determine whether a similar incidence of *ras* mutations can be observed in pediatric patients. 21 children with CMML aged 5 months to 6 years were examined at various stages of disease progression. Neurofibromatosis type 1 and monosomy 7 were known in one patient each. Genomic DNA was obtained from myeloid cells of peripheral blood (n=11), bone marrow (n=8) or spleen (n=1) or from mononuclear bone marrow cells (n=1). To analyse codons 12 and 13 of N-*ras* and K-*ras*, polymerase chain reaction (PCR) with mismatched primers was performed in order to introduce artificial restriction sites. The PCR products were screened for mutations through restriction fragment length analysis. Loss of cleavage site could be observed in 4 patients, located in codon 12 of N-*ras* (n=1), codon 13 of N-*ras* (n=1), both codons 12 and 13 of N-*ras* (n=1), and codon 13 of K-*ras* (n=1). The base exchanges are currently defined by direct sequencing. For codon 61 of K-*ras*, direct sequencing of exon 3 was performed on samples of 12 patients. No base exchange was detected. Codon 61 of N-*ras* and codons 12, 13 and 61 of H-*ras* are currently under investigation. Our preliminary results may indicate that *ras* mutations are less frequent in children with CMML than in adults.

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FAVOURABLE RESPONSE OF CHRONIC MYELOMONOCYTIC LEUKEMIA TO INTENSIVE CHEMOTHERAPY

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We present the results of three patients diagnosed of chronic myelomonocytic leukemia (CMML) who have been treated with the BFM-87 protocol for AML. None of them had an HLA identical related donor. The mean age at presentation was 30 months (range from 23 to 33). Two of them had persistent CMV and EBV infection at diagnosis. Clinical features and hematologic findings were characteristic of CMML: hepatosplenomegaly, anemia, leukocytosis (neutrophylia, monocytosis, and presence of blast cells) and trombocytopenia. HgbF level was increased in one case (5%).
The bone marrow examination showed:

Pt	*Cell*	*M/E*	*Disp*	*Bl* %	*Mono* %	*Eos* %	*Cytogenetic*
1	"	4/1	+++	5	-	-	46,XY q$^+$
2	"	12/1	++	11	15	21	45,XY, t (15;17)✡
3	"	7/1	++	5	28	3	46,XX

✡ 45,XY, t (15;17) (q22;q12), -17, -20'der (20), t (17;20) (q21; p12)
The immunophenotype was studied in patients 2 and 3 exhibing 13% and 24% of monomacrocytic lineage and 6% and 8% of myeloblastic cells with monocytic markers. Cell cultures from peripheral blood and bone marrow showed spontaneous growth of monocyte-macrophage colonies in all of them. The three patients were treated with intensive chemotherapy (IC) according to BFM-87 protocol for AML; only pt 2 and 3 achieved complete response; pt 1 continued treatment with mild chemotherapy (low-dose ara-C) with partial response. The medium follow-up is 30 months (15-38 mo) and the three patients are alive and asyntomatic. We conclude that the t (15;17) translocation is not an adverse prognostic factor in childhood CMML since pt 2 is in clinical, cytogenetic and immunologyc remission for 35 months.

P-53

AUTOIMMUNE PHENOMENA AND CHILDHOOD MYELODYSPLASIA

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Autoimmune disorders (SLE, autoimmune thrombocytopenia, vasculitis, presence of antinuclear antibodies - ANA, antineutrophil cytoplasmatic antibodies-ANCA and other), have been mentioned in primary myelodysplastic syndromes (MDS). The autoimmune MDS, without cytogenetic anomalies and no further clonal evolution, have been also described as transient disorders (2% of total MDS).
Autoimmune disorders have been revealed in 3/20 of patients with MDS, all females, aged from 19 mo to 7 yr. They all had pancythopenia and signs of autoimmune disease. Diagnosis of MDS has been established on the basis of hematological examination and confirmed by bone marrow histopathology. Cytogenetic analyses revealed karyotype abnormality in 1/3. In the patient M.T. with hypoplastic MDS (myelofibrosis with RAEB-t M7, no cytogenetic abnormality), the hematological disorder has been associated with presence of ANA, low levels of complement, mesangioproliferative glomerulonephritis and evolution toward nephrotic syndrome. In the patient S.Lj. the diagnosis of MDS with monosomy 19 has been established concomitantly with the diagnosis of coeliac disease. Third patient JB. has had MDS allied with cutaneus vasculitis, which periodically deteriorate paralelly with changes of her bone marrow and peripheral blood findings. Autoimmune disorders that emerge during the evolution of MDS are usually regarded as nonspecific. In fact, being familiar with this phenomena is important because autoimunne diseases could mask typical findings of myelodysplastic syndrome.

P-54

MONOSOMY 7 IN CHILDREN WITH MYELOPROLIFERATIVE DISORDERS

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Monosomy 7 has been discovered in 6/30 or 20% children, four boys and two girls, aged from 5,5 mo to 11 yr, with myeloproliferative diseases. In five, monosomy 7 has been confirmed as *de novo* disorder and in one in association with neurofibromatosis type 1 (NF-1) and congenital heart disease. In this child, monosomy 7 and del 4 (q31) has been revealed at the time of diagnosis of acute leukemia (AML-Mo). In children with *de novo* disorder, monosomy 7 have been associated with MDS in four, and with juvenile chronic myeloid leukemia (JCML) in one, during the clonal evolution of his disease. In one case the familiar occurrence of myelodisplastic syndrome and aplastic anemia has been confirmed. In this group of patients no secondary form of monosomy 7 has been confirmed. The patients with MDS and monosomy 7 had have an aggressive course. Two of them evolueted toward AML M6 , two months and one year respectively, after the diagnosis of MDS. Conventional chemotherapy (BFM 87) was unsuccessful in children with AML. They died because of the underlying disease as did the patient with JCML. Two children with monosomy 7 are alive. In a boy with one year long history of anemia and in the other followed for rheumatoid arthritis and anemia during 9 months, the diagnoses of myelodisplastic syndrome with monosomy 7, have been established and an allogenic bone marrow transplantation from HLA identical sibling has been performed in a short period of time after the diagnosis of MDS.

P-55

CLINICOPATHOLOGICAL FEATURES OF CHILDREN WITH Ki-1-POSITIVE ANAPLASTIC LARGE-CELL LYMPHOMA
CENTRAL NERVOUS SYSTEM INVOLVEMENT IN TWO CASES

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Ki-1-positive anaplastic large-cell lymphomas [ALCL] have some particular clinical features. While extranodal localizations are common, initial CNS involvement has been virtually not reported and meningeal recurrence is also extremely rare. Here we report the clinicopathological features of *9 patients with ALCL,* treated at the Emma Children's Hospital from 1990 to 1995, *of whom two had CNS disease.*
Characteristics of patients (n=9): age:1,5 to 13 year (median 9 year); 5 M/4 F; stage I n=1, st II n=3, st III n=3, st IV n=2; sites of disease at presentation were: lymph nodes in 8, skin in 2, CNS, bone, lung, liver, spleen in 1-1 pts resp.; no bone marrow infiltration has been found. Tumor cells were of T-cell origin in all cases. From six patients where cytogenetical study succeeded three had translocation t(2;5)(p23;q35). Seven patients have been treated uniformly with an intensive chemotherapy of one year. One patient (initially st.II lymphnode sites) experienced a recurrence 15 month after dg with multiple lymphnodes and extranodal (CNS, lung, skin) sites. She died of infection in 2nd CR 26 month after the dg. All the other patients are in 1st CR with a follow-up time of 2 to 62 months (mean: 26 mo). *Both children with CNS involvement, one at the diagnosis and one at the recurrence, had at that moment an advanced disease with multiple nodal and extranodal* localizations.
CONCLUSION: Our results are consistent with the data on the specific clinical presentation of the disease and indicate that *CNS involvement can occur in children with ALCL even in patients with an initially localized disease.* Treatment results are satisfying.

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DURAL SINUS THROMBOSIS IN CHILDREN WITH CANCER

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Dural sinus thrombosis (DST) has been reported in association with cancer in adults and children. The objective of this study was to establish the frequency, predisposing factors and prognosis of DST in a population of children with solid tumours.

Between 1980 and 1995 DST was diagnosed in 7 children in our centre. Six of them were boys. Median age was 13 years (range 8-15). Six children were being treated for non-Hodgkin lymphoma and 1 for neuroblastoma. The main presenting symptom was the occurrence of seizures, present in all patients. The diagnosis was confirmed by cerebral CT scan in 6, by MRI in 2 and by angiography in 4 cases.

In 2 children the probable cause of DST was found. Dehydration in combination with a poor general condition seemed to be the cause in the patient with neuroblastoma. Tumour localisation in the CNS probably caused DST in 1 patient who was treated for Ki 1 lymphoma.

The cause of DST was not clear in 5 children with non-Hodgkin lymphoma (3 stage IV lymphoblastic lymphoma and 2 stage III Burkitt's lymphoma). In these patients DST occurred early in the course of therapy. The median interval between start of therapy and onset of symptoms was 19 days (range 8-40). The maximum duration of symptoms was 5 days. The children were all in a good general condition. Chemotherapeutic treatment was tolerated well. No evidence of tumour could be found at time of onset of symptoms related to DST. No child suffered from thrombocytopenia or thrombocytosis. There was no relation with the administration of any specific drug.

We conclude that dural sinus thrombosis is a rare event occurring in children with cancer. It almost exclusively occurs in children with advanced stage non-Hodgkin lymphoma, particularly within the first 2 months of therapy. In the event of seizures in children treated for non-Hodgkin lymphoma this diagnosis should be considered immediately after establishing the absence of metabolic disturbances. The prognosis is good and no specific therapy is necessary. A detailed study of haemostasis and fibrinolysis in children treated for non-Hodgkin lymphoma should be performed in the search for an explanation of this complication.

P-57

UNSUCCESSFUL TREATMENT OF RELAPSING HIGH-GRADE NON HODGKIN'S LYMPHOMA (NHL) WITH "VIP" REGIMEN FOLLOWED BY HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE

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From the early eighties, we adopted for relapsing children with high grade NHL the "VIP" regimen consisting of Cisplatinum (25 mg/sqm/d for 4 days), Ifosfamide (1.5 g/sqm/d for 4 days), Etoposide (200mg/sqm/d 2 days apart) and Desametazone (8 mg/sqm/d for 2 wks). Up to 1992, 18 children were thus treated either at their first (11) or second (7) relapse. Eleven of 18 pts (61%) obtained remission (8 CR+3 PR) for a median time of 4 mos (range 1-14 mos). After these preliminary results, with the aim to maintain remission in pts without bone marrow sibling donors, consolidation and eradication regimens were added following two VIP courses. These consisted of Citoxan (7g/sqm in 5 doses) on day 0, Methotrexate (8g/sqm 6-hour infusion)+Vincristine (1.4 mg/sqm ev) on day 28, Etoposide (2g/sqm 10-hour infusion) on day 42 followed by Cytarabine (1g/sqm/d continous infusion for 4 days+0.5 g/sqm/d ev for 4 days) plus Cisplatin (20 mg/sqm/d continous infusion for 4 days) on day 70. After HD-CTX and VP16, s.c. G-CSF 5 mcg/kg/die was prescribed and mobilized circulating hemopoietic progenitor cell (CDC) harvest was performed when possible. Between March '93 and June '95, 10 pts with St. Jude's stage 3/4 NHL (5 T-lymphoblastic, 3 ALCL, 1 centroblastic, 1 common LLA) were treated at first relapse: 4 CR+2 PR were obtained after two VIP courses, while NHL rapidly progressed in 4 children. Four of 6 completed the whole chemotherapy program; 5/6 could be submitted to CDC harvest and no one required hemopoietic rescue. All of them eventually died of their disease with a median progression-free survival of 6 mos. The whole program was performed in a mean of 150 days because of the long interval between courses due to severe bone-marrow toxicity. This intensive high-dose treatment program, besides being very expensive in terms of hospital stay and supportive care, failed to obtain long-lasting remissions, thus prompting us to investigate new second-line treatment for relapsing NHL children.

P-58

FAVOURABLE OUTCOME AFTER ONE YEAR TREATMENT OF CHILDHOOD T-CELL LYMPHOMA/T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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For T-malignancies in children a poor prognosis is reported. In these malignancies a combination of lymphoma and leukemia is commonly seen at presentation and most patients are treated according to protocols for acute lymphoblastic leukemia (ALL). In pediatric lymphoblastic non-Hodgkin's lymphoma without bone marrow infiltration various protocols have been used. The most frequently reported LSA_2-L_2 regimen (and its modifications) shows variable survival rates between 40 and 75%. From 1989 we have treated 21 consecutive patients with T-cell malignancies, irrespective of localisation, with a protocol consisting of a 4 agent induction treatment followed by high doses of methotrexate and cytosine-arabinoside and intensified BACOP courses. Treatment duration for each patient was one year. Fifteen patients had stage IV disease. Follow-up ranged from 2 to 6 years (median 3.5 years). Overall event-free survival (EFS) was 81%, whilst in those with stage IV disease this was 74%. No therapy related deaths occurred. As prognostic factors, only a WBC > 100 x 10^9/L was marginally significant with respect to survival ($p = 0.06$). Evaluation of toxicity revealed a minimal decrease of CO-diffusion and cardiac shortening fraction. We conclude that a relatively short, but intensive chemotherapy can be used in T-cell malignancies. The EFS is satisfying, but larger studies are needed.

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SUCCESSFUL OUTCOME IN THE TREATMENT OF CHILDHOOD ANAPLASTIC LARGE CELL LYMPHOMA Ki-1(CD30)$^+$.

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We retrospectively reviewed the outcome of children treated for primary Ki-1(CD 30)$^+$ T-cell lymphoma in the Gauteng Area, South Africa. Seven children were diagnosed from 1990-1995, with a median aged of 11.2 years. Male:Female ratio was 1:1.3. All patients had a T-cell immuno-phenotype and strong reactivity to CD30 monoclonal antibody. Clinically most patients presented with significant adenopathy (6 patients), 3 patients with "B" symptoms, 3 with radiological bony changes and skin involvement in 1 patient. Only 1 patient had bone marrow involvement. Three patients were treated with a long course (2 years) of chemotherapy. - The Berlin High Risk Protocol. Four patients on a short pulse (6 months) chemotherapy - Berlin B Protocol. No radiation was received. Remission was achieved in all patients by day 21 - 48 (mean 29 days). Both protocols were tolerated with minimal toxicity. All patients remain in first complete remission (CR) for a median time of 1 year 4 months (range 2 months- 45 months). Both short pulse and long maintenance B-cell directed chemotherapy have been effective in the treatment of Ki-1 lymphomas at our institutions.

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NON-HODGKIN'S LYMPHOMA: RESULTS OF MCP-842 PROTOCOL

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Non-hodgkin's lymphoma (NHL) in children are of diffuse histology and are characterised by wide dissemmunation and rapid progression. The aimmof this study was to treat patients with an aggressive protocol of short duration with radiation. 75 previously untreated patients, were enrolled on MCP-842 protocol f rom Aug'86 to Dec'92 and result are as per analysis on Feb'95. Fifty five were male and 20 were female and 61 patients belongs to high risk category. Thirty nine (52%) were diagnosed as lymphoblastic lymphoma (LL) and 18 case as diffuse large cell lymphoma(DLCL)and small non cleared cell lymphoma(SNCCL.)The treatment protocol comprise of A and B cycle of 8 non cross resistant drugs. Sixty seven(89.3%) achieved complete remission(CR). There were 15 relapse (10 LL, 2 DLCL and 3 SNCCL). Five of the 10 relapse among LL were in patients with mediastinum as primary. At the age of 6 years, the event free survival was 56.81% (41%,70% and 71%) for LL, SNCCL abd DLCL respectively). The disease free survival was 71.38%. The clinical implications will be discussed.

Key Words: NHL, SURVIVAL

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SERO-EPIDEMIOLOGICAL STUDIES RELATED TO EPSTEIN-BARR VIRUS AND HTLV-1 IN HEALTHY TURKISH CHILDREN AND IN MALIGNANT LYMPHOMA PATIENTS

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Acute leukemias and malignant lymphomas (ML) are the 2 most common malignant diseases in pediatric age group in Turkey, which is a developing country. This study represents the sero-epidemiologic studies related to Epstein-Barr virus (EBV) and HTLV-1, to define their role in the etiopathogenesis of malignant disease. In EBV study group 120 children with ML namely 51 Hodgkin's lymphoma (HL) 36 Burkitt's lymphoma (BL) 33 non-Burkitt, non Hodgkin's lymphoma cases were enrolled. There were 66 males, 54 females, with an age range of 2-14 years. In HD group (M/F:40/11) median age was 8 years with 64% of them having mixed cellular histological subtype. In BL group (M/F:22/11) median age was 5 years whereas in non Burkitt's group (M/F:24/9) median age was 9 years. As far as the frequency of EBV infection is concerned, 94% of HD cases, 91.6% of BL cases and 78.7% of non Burkitt's patients were found to be seropositive, respectively. Among healthy population, 311 children from different age groups were examined. In the age group of 6-18 months 41.6%, 2-6 years of age 69% and 7-15 years of age 81.8% of the children were found to be seropositive for EBV antigents, respectively. In HD and BL group the frequency of EBV infection was significantly higher when compared to non Burkitt's and control groups. The highest antibody titers to EBV were determined in BL (anti-EBV-VCA 1/300, anti-EBV EA 1/204, EBNA 1/393) and in HD (anti-EBV-VCA 1/157, anti-EBV EA 1/99, anti-EBNA 1/100) patients accordingly. These results were higher than control group (anti EBV-VCA 1/93, anti-EBV EA 1/56, anti-EBNA 1/49) and non Burkitt's group (anti-EBV-VCA 1/109, anti-EBV EA 1/53), respectively. The frequency of HTLV-1 seropositivity were carried-out in 18 ALL (6 T-ALL, 12 non-T ALL) and 23 ML (6 lymphoblastic, 10 HL, 7 non-BL, non HL) cases. The seropositivity to HTLV-1 was only detected in a 5 year-old-boy with T-ALL. In control group for HTLV-1 20 healthy children and adults whose ages ranged from 4-40, there were no seropositivity. As a result, this study indicate that healthy Turkish children are exposed to EBV infection commercing from early ages of their lives. A growing body of evidence suggests that non endemic BL, but type I epidemiologic pattern of HL, which is common in Turkey, as well is strongly associated with EBV infection. We believe that more detailed studies, directed to predisposing cofactors in addition to EBV and other environmental features are required in order to assess risk of developing ML. Limited number of cases studied for frequency of HTLV-1 infection showed that this type of infection is not a common problem in Turkey.

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HUMAN HERPESVIRUS 6 (HHV-6) ASSOCIATED WITH KAPOSI'S SARCOMA IN A CHILD POST AUTOLOGOUS BONE MARROW TRANSPLANT (BMT) FOR ANAPLASTIC LARGE-CELL LYMPHOMA.

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Kaposi's sarcoma (KS) commonly occurs in human immunodeficiency virus (HIV) infection, but is rare post BMT with only two previous cases reported. Epidemiological studies have suggested an infectious cause for KS; recently herpesvirus-like DNA sequences have been demonstrated in KS tissue of patients with and without HIV infection, as well as in HIV-associated lymphomas. Later studies corroborated the presence of a new herpesvirus now called Human Herpesvirus 8 (HHV-8). HHV-6 has also been found in KS tissues of HIV infected individuals but was dismissed as a causative agent because the virus was detected in the uninvolved surrounding skin. We describe a 3-year-old boy with recurrent anaplastic large-cell lymphoma (ALCL) who developed multicentric KS post autologous BMT. We performed PCR analysis on this patient's KS tissue (lung and skin) and ALCL tissue (diagnosis and relapse), and on 6 ALCL cell lines for the presence of HHV-6 and HHV-8 DNA sequences. Using specific primers and positive controls, HHV-6 DNA sequences were found in both the KS tissues and in the relapse ALCL samples, but not in the initial ALCL tissue, nor the 6 ALCL cell lines analyzed. No HHV-8 DNA sequences were found in any of the specimens. Our results indicate that HHV-6 infection may have a role in the development of KS post BMT. The lack of HHV-6 and HHV-8 DNA sequences in the ALCL tissues (HHV-6 in the relapse tissue only) and cell lines argue against a pathogenic role of HHV-6 and HHV-8 in ALCL.

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MYELODYSPLASTIC SYNDROME AND ACUTE LEUKEMIA AFTER TREATMENT FOR OSTEOSARCOMA

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Five-hundred and twenty pediatric patients with osteosarcoma have been treated with a cis-diamminedichloroplatinum-II (CDP) based regimen. Among these patients, five developed acute leukemia and one myelodysplastic syndrome. In addition to CDP, other agents utilized in the treatment of these patients and the associated hematologic complications were as follows: acute leukemia: High dose methotrexate-leukovorin (MTX-LF) and Adriamycin (ADR) (2 patients); MTX-LF, ADR and Ifosfamide (IFX) (1 patient) and

MTX-LF, ADR, IFX and Etoposide (VP-16) (3 patients). There were 4 girls and 2 boys. The age at diagnosis varied from 10-21 years. The median interval between termination of treatment for osteosarcoma and onset of secondary disease was 9 months (range, 1-28 months). Survival after onset of secondary disease ranged from 2 days to 2 years. Exposure to alkylating agents, CDP and VP-16 has been implicated in development of secondary leukemia. In contrast, osteosarcoma per se is not a predisposing factor in the development of acute leukemia or other myelodysplastic syndromes. The appearance of these complications in 6 of 520 treated osteosarcoma patients early after completion of therapy is of major concern. The putative offending agents may be operative individually or in combination. Additional reports of similar complications in treated osteosarcoma patients and further studies are necessary to clarify the mechanism of action of this form of carcinogenesis.

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INCREASED INCIDENCE OF SECOND LEUKEMIA AFTER MAINTENANCE CHEMTOTHERAPY IN NEUROBLASTOMA PATIENTS

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Patients with embryonal tumors may be considered genetically at risk to develop second malignancies. In neuroblastoma, the observed incidence was rather low until recently (6 cases per 1465 patients). Here we report an unexpected increase of myeloproliferative diseases in high risk neuroblastoma patients exposed to maintenance chemotherapy.

Out of 70 patients with completed maintenance chemotherapy according to the trial NB90 GPOH, 5 developed a second malignancy (3x AML, 1x CMML, 1x RAEB). 4 patients had stage 4 neuroblastoma with bone marrow involvement and were 24, 25, 32 and 43 months at diagnosis, 1 patient had stage 3 (33 months) at diagnosis. The second leukemia was diagnosed 35, 18, 17, 7 resp. 1 month after the end of maintenance therapy. 2/5 patients showed severe bone marrow hypoplasia during maintenance chemotherapy. Of 4 available cytogenetic examinations, one 4,11 translocation was found. 2 patients are alive, 3 died from septical complications during chemotherapy.

No second malignancy was observed in the 47 high risk neuroblastoma patients who underwent megatherapy with autologous stem cell rescue.

In view of the identical induction chemotherapy in both arms we speculate that the prolonged exposure to oral etoposide may contribute to the development of secondary myeloproliferative disorders. As a preliminary consequence oral etoposide was omitted from the maintenance chemotherapy arm.

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LEUKAEMIA PHENOTYPE AND ETHNICITY IN CHILDREN LIVING IN THE UNITED ARAB EMIRATES

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Childhood lymphoid malignancies have a different distribution in industrialized countries to those of developing countries. We have previously shown that the relative frequency of acute lymphoblastic leukaemia (ALL) in children of Subcontinental origin living in the UAE was significantly higher than in children of local UAE or other Arab origin.

Immunophenotyping was started on all newly diagnosed ALLs in 1994. We present here the early data relating to the phenotype and ethnicity in these patients. Thirty-two newly diagnosed patients had their presentation bone marrow cells phenotyped. Ten of these patients had T-cell precursor leukaemia, 22 had B-cell precursor ALL. Clinical findings reflected the well known differences between the two phenotypic groups. No difference was found in the proportion of patients with hyperdiploid chromosome number between the two groups.

On the basis of our previous studies it was expected that children of Subcontinental origin would have predominantly common ALL. In fact when the distribution of phenotype among the three ethnic groups was analyzed, children of Subcontinental origin had a significantly higher proportion of T-cell cases than the other two ethnic groups.

The present preliminary data suggest that the immunophenotypic pattern in Subcontinental children living in the UAE is not different from that described in their home country. It seems likely, that with improving socioeconomic conditions the relative incidence of lymphomas decreases, giving rise first to a higher proportion of T-cell leukaemias, followed later by the typical peak of CD10+, B-cell precursor ALL.

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INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) IS LOW IN MALNOURISHED CHILDREN WITH SOLID TUMOURS

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In children with solid tumours, tumour weight may account for up to 7% of the child's total weight. Weight and related parameters, therefore may be poor indicators of nutritional status. As IGF-1 and its binding protein (IGFBP-3) are influenced not only by growth hormone status but also by nutrition, we have assessed the relationship between these two peptides and auxiological parameters at presentation in 26 children with solid tumours, [median age 4.3 (0.5 - 15.75) yrs.,11 male, 4 pubertal]. Weight (WT), height (HT), skinfold thickness (SFT) and mid-upper-arm circumference (MUAC) were measured at presentation. Malnutrition was defined as WT/HT < 85% and/or SFT/MUAC < 3rd centile. Serum IGF-1 and IGFBP-3 were measured by radioimmunoassay and expressed as standard deviation scores (SDS). The median SDS for WT, HT and WT/HT were -0.43, -0.23 and +0.08 respectively which were not significantly different from normal. In contrast the SDS for MUAC, subscapular and triceps SFT were -1.25,-1.43 and -0.93 which were significantly different from normal ($p<0.001$). The median SDS for IGF-1 and IGFBP-3 were -1.37 ($p<0.001$) and +0.1 ($p=0.8$) respectively, with strong correlation between the two ($r=0.7$, $p<0.001$). IGF-1 and IGFBP-3, however, did not correlate with weight and height indices but did with MUAC ($r=0.4$, $p=0.03$ for IGF-1) and with subscapular SFT ($r=0.44$, $p=0.03$ for IGF-1 and $r=0.4$, $p=0.04$ for IGFBP-3). In addition, malnourished children had a low IGF-1 SDS compared to the levels in the nourished, (-2.4 vs. +0.06, $p=0.02$) but not for IGFBP-3 (-0.04 vs. +0.45, $p=0.37$). We conclude that IGF-1 is lower in children with solid tumours who are malnourished at presentation. At this stage these peptides reflect nutritional status rather than growth, while weight, height and weight related indices are unreliable for assessing nutritional status.

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TYPHLITIS - AN UNUSUAL COMPLICATION OF NEUTROPENIA

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Typhlitis is a necrotizing enteropathy of the bowel wall affecting the cecum that may result in perforation and occurs only in the setting of severe neutropenia. It is usually underdiagnosed and tends to have a fatal outcome.
This study analysed the clinical, laboratorial and radiological features of paediatric patients diagnosed with Typhlitis at our hospital between 1993 and 1996. There were 7 males aged from 3 to 16 (median 7). Six patients were receiving treatment for acute lymphoblastic leukemia, and one acute myelogenous leukemia. All of them had received either induction or intensification chemotherapy in the preceding weeks. Four patients had gastrointestinal tract bleeding; four had abdominal distension, muscular guarding and rebound tenderness; three had diarrhoea. The leukocyte count ranged from 100 to 500 (median 100) and the platelet count was below 20.000 in all patients. The abdominal ultrasounds showed concordance with the abnormalities described in the x rays, which were bowel wall thickening in 5 patients, loop distension in 4 and free peritoneal liquid in one. Blood cultures were positive for *Escherichia coli* in two patients and for *E. coli* and *Sthaphylococcus aureus* in one patient. All patients received conservative treatment consisting of bowel rest, parenteral broad-spectrum antibiotics, analgesics and parenteral fluids. One patient underwent surgery and was submitted to right hemicolectomy. Two children died from sepsis. The clinical presentation of typhlitis in our sample were similar to the literature, but mortality (40%) was inferior than described in other studies (50% to 100%). In our experience, conservative management with frequent surgical reassessment was the safest way to identify the uncommon patients who may benefit from surgery.

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WHEN SHOULD THE VACCINATION AGAINST HBV INFECTION BE APPLIED IN CHILDREN WITH LEUKEMIAS

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Hepatitis B virus (HBV) infection are a serious epidemiologic problem especially in the "high risk" patients.It is maintained that the only effective way of prevention is the active prevention.
Engerix B (Smith Klein-Beecham) vaccination has been applied in 108 children with leukemias who were qualified to three different groups.

In 42 children vaccination has been performed in an accelerated system -0-10-20 days at the first stage of the disease during intensive chemotherapy. Remaining patients have been vaccinated according to the pattern 0-1-2-6 months. Thirty two children were vaccinated during the maintenance treatment and 33 - after its completion. In all cases due to the immunosuppressive treatment the children were vaccinated with a double dose suitable for their age.

The obtained level of anti-HBs above 1000mIU/ml was recognized as a very good reponse, above 100mIU/ml as good and above 10mIU/ml as satisfactory. The level of anti-HBs below 10mIU/ml was regarded as no response.

The results of vaccination performed at the first stage of the disease during the intensive chemotherapy were unsatisfactory. In 70.7% of children the response was mild and in 19.5% good but close to the lower border and in 9.8% of children lack of response to the treatment was observed. In one child the presence of anti-HBs antigen after the completion of the vaccination was determined. In children vaccinated during the maintenance treatment the results were also discouraging. Very good results were obtained only in 25% of children, good results in 9.3% satisfactory in 31.3% and in 34% lack of response was determined. After the vaccination carried out in the group of children who had completed chemotherapy the obtained results were good. In 54.5% of patients the response was very good, in 30.3% good and in 15.2% mild. In 12 children with no response a repeated vaccination cycle was performed according to the same pattern. In 7 children the obtained results were either very good or good. In one case no protevive level of antibodies has been obtained.

Our studies proved low efficacy of the vaccination applied in children with leukemias during the chemotherapy. It is necessary to apply passive immunity during chemotherapy and to carry out the vaccination after the completion of chemotherapy in order to prevent the HBV invection.

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TROPISETRON IN THE CONTROL OF NAUSEA AND VOMITING INDUCED BY COMBINED CANCER CHEMOTHERAPY IN CHILDREN

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Tropisetron (Navoban, Sandoz Pharma Ltd.), a selective antagonist of the serotonin receptor (5HT3), was evaluated in the prevention of chemotherapy-induced nausea and vomiting in 40 children with miscellaneous malignancies (Age range: 6 months-15 years). The most common malignancy (% 30) was acute lymphocytic leukaemia.

Patients received tropisetron during two or more courses of emetogenic chemotherapy for a total of 140 courses administered iv (% 66) or iv+it (% 34). Twenty four chemotherapy courses (% 17) included cisplatin. Tropisetron (0.2 mg/kg/day, maximum: 5 mg/day) was administered as a single iv dose slowly, before the start of chemotherapy on day 1 and iv or by mouth the subsequent days (median treatment duration: 5 days).

Overall complete response (absence of both nausea and vomiting) on day 1 was observed in 118 out of 140 chemotherapy courses (% 84). The patients receiving cytotoxic chemotherapy had a % 77 complete response rate and a % 26 partial response (one to four vomits and/or less than 5 hours of nausea) rate during the first 24-hour period of the first course.

We observed headache (2 cases), diarrhea (1 case) and loss of appetite (1 case) as side effects. The adverse effects were mild and did not lead to discontinuation of treatment.

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DEVELOPING PAEDIATRIC PALLIATIVE CARE EQUIPMENT CASES

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Following many paediatric palliative care experiences in the home setting, the West Midlands Paediatric Macmillan Service (WMPMS) have identified a need to implement the provision of palliative care more efficiently in this setting.

Specific problems identified include :

1. The often rapid change in a child's symptoms requiring new drugs or a change in administration route.

2. Difficulties of access to appropriate drugs and equipment out of office hours.

3. The large geographical area covered by the WMPMS, with families living up to 80 miles from the Regional centre.

The WMPMS have in the past collaborated with both families and local primary health care teams to ensure that a variety of drugs and equipment have been available in the home in anticipation of the potential need for IV/SC access and dosing. This, however, has always been an informal, nurse-led initiative, relying upon the individual skills of nurses to secure the necessary resources.

The WMPMS, together with the pharmacy department at Birmingham Children's Hospital (BCH) decided to standardise their approach by developing palliative care equipment cases containing the IV/SC drugs and equipment most likely to be needed in the home situation. These will be used together with specific community prescription sheets and terminal care guidelines developed at BCH to ensure that the specific problems highlighted above are addressed.

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CHARACTERISTICS and CLINICAL OUTCOME of FEBRILE EPISODES IN NEUTROPENIC ONCOLOGY PATIENTS

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In this prospective single institution study, frequency, characteristics and treatment results with three different empirical antibiotic regimens of febrile episodes in 102 neutropenic patients receiving intensive cytotoxic chemotherapy were evaluated. From May 1994 to February 1996, 40 children with leukemia/lymphoma and 18 children with solid tumors, 34 males and 24 females, ranging in age from 10 months to 14.5 years, had fever > 38.5 C and neutropenia < 500/µl on 115 occasions. The neutropenia was profound (< 100/µl) in 60.6%. The mean duration of neutropenia was 8.2 (2-35) days. Microbiologically documented infections occured in 56 episodes (48.6%), only clinically documented infection, pneumonia being the most frequent, on 9 other and only severe stomatitis on 11 other occasions. Frequency of gram negative and positive bacteria isolated were identical (50%), S.viridans and S.aureus being the most frequent G (+) and E.coli and Klebsiella spp. being the most frequent G (-) pathogens. Blood cultures were positive in 24 episodes (20.8%) in 15 which (62.5%) the causative organism was G (+) , primarily S. Epidermidis and S. Aureus This was associated with the presence of a central venous catheter in only one patient. Multiple bacteria grew in 17 episodes. Mycotic infections alone or mixed with bacterial infections were found in 10 episodes. The patients received one of three empirical antibiotic regimens: A, piperacillin+cefazolin+netilmicin (27 cases); B, cefoperazone-sulbactam + netilmicin (42 cases); C, imipenem/cilastatin (46 cases). Total response rate within three days was 53.9%; while it was 52%, 36% and 72% and percentage of profound neutropenia was 37.5%, 65.6 % and 69.7% in the three groups respectively. The mean duration of fever was 4.0 days, 3.4, 3.8 and 4.9 days in groups A,B and C. Modifications were required in 59%, 62% and 54% of the episodes with on average of 9.9, 8.3 and 9.4 days of antibiotic use in groups A,B and C respectively. There were 5 cases of infection related deaths, three due to G (-) bacteremia (P.aeruginosa and E.coli) and one due to systemic Candida infection. All of the deaths occured in patients with active underlying malignancy and profound neutropenia, which seem to be the main risk factors.

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THE USE OF CENTRAL VENOUS CATHETER IN PAEDIATRIC HAEMATOLOGY / ONCOLOGY PATIENTS

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A 2-year retrospective survey of the use of central venous catheters (CVC) was performed to review its use and complications. 47 CVCs (27 single-lumen catheters, 17 double-lumen catheters and 3 subcutaneous ports) were placed in 38 consecutive patients (age range 0.49-19, median 4.85 year). All except 2 CVCs were inserted through jugular veins. The indications were for standard-dose chemotherapy in 23 (49%), bone marrow transplantation 18 (38%) and other 6 (13%). The total lifespan of the CVCs was 8042 (median 149, range 9-572) days. The rate of documented catheter-related bacteraemia was 3.4 episodes /1000 catheter days with a total of 28 episodes. The infective agents were grouped under the following categories, *Staphylococcus aureus* and *Coagulase negative staphylococcus* (13), *Bacillus cereus* and other species (11), Non-fermenters (11), *Enterobacteriaceal & Vibrionaceal* (3), and *Streptococcus sanguis* (1). The three most common organisms were *Coagulase negative staphylococcus, Staphylococcus aureus and Bacillus cereus*. The reasons for removal of CVC were cessation of indication in 13 (27%), death 8 (17%), catheter-related bacteraemia 8 (17%), exit site infection 7 (15%), dislodgment 7 (15%) and internal rupture 1 (2%). Three (7%) CVCs were still in use at the end of the study. Catheter-related infection remains the most important complication and the leading cause of CVC removal.

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THE IMPACT OF GRANULOCYTE COLONY-STIMULATING FACTOR PROPHYLAXIS IN NEUTROPENIC CHILDREN TREATED WITH CONVENTIONAL CHEMOTHERAPY FOR NON-MYELOID MALIGNANCIES

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Object of study: This retrospective study was conceived and conducted in order to look into the impact of prophylactic granulocyte colony-stimulating factor (G-CSF) administration on paediatric cancer patients with conventional chemotherapy-induced neutropenia complicated by fever and/or infection.

Methods: We reviewed a total of 244 episodes of chemotherapy-induced neutropenia in 104 children with various non-myeloid malignancies. In 214 episodes (81 study patients) G-CSF had been prophylactically administered at a dose of 5 µg/kg per day, for 7-10 consecutive days, starting 24 hours after stopping chemotherapy. These patients were compared with 23 historic controls (30 neutropenic episodes) who had received either identical or similar drug combinations.

Results: We found statistically significant diffenrences in all parameters investigated. Neutropenic nadirs lasted longer in the control group than they did in the study group (interval required for neutrohil recovery to levels of ≥ 1,000/mm^3, 8.53±2.93 vs 3.92±2.33 days, p<0.001). Neutropenic fever rate (35% vs. 86.7% of courses, p<0.00001) and duration (1.54±2.93 vs. 5.7±4.82 days, p<0.001) were also found reduced in the study group. Also the frequency of documented infections (6.1% vs 26.6%, p=0.00017) and that of overt mucositis (6.1% vs 46.6%, p<0.00001) were lower in the patients who received G-CSF. Finally there was a shortening in hospital stay (4.01±5.4 vs.10.93±5.92 days, p<0.001) as well as a reduction in antibiotic consumption (3.16±4.91 vs 9.53±5.92 days of antibiotic treatment, p<0.001 and 35% vs. 83.3% of the courses, p<0.00001) in the study group as compared with the control group.

Conclusions: From evaluating our results it appears that prophylactic G-CSF administration in children receiving conventional but intensive chemotherapy for non-myeloid malignancies, induces a singnificant reduction in days of neutropenia, neutropenic fever, documented infections and mucositis rates, as well as hospitalization duration and antibiotic consumption.

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EFFECT OF FILGASTRIM IN THE PREVENTION OF CHEMOTHERAPY-INDUCED MUCOSITIS IN CHILDREN WITH NON-HODGKIN'S LYMPHOMA.

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Intensive polychemotherapy regimens have contributed to the increasing survival rate of children affected by non-Hodgkin lymphoma. This therapeutic approach is followed by severe effects among them myelosuppression and mucositis which can interfere with the therapeutic compliance.
Objectives: To evaluate the eficacy of filgastrim in the reduction of episodes and severity of chemotherapy-induced mucositis.
Material and Methods: From January 1991 to January 1996, 47 pts with non-Hodgkin lymphoma were treated with LMB89 and LMT89 protocols according to Working Formulation histopathological classification. Pts were distributed into two groups that differed only in prophylactic use of filgastrim during the induction phase. In group 1, 23 pts (14 M, 9 F) aged 30 m-15 y , received chemotherapy without filgastrim support. The 24 patients in group 2 (14 M and 10 F) aged 27 m-16 y received the same chemotherapy regimens plus filgastrim (5 ug/Kg) from the end of CT to neutrophil recovery.
Results: The incidence of severe mucositis (grade III, IV) was uncommon in both groups (1 pt in both groups). Mucositis grade I, II was present in only 2 pts of group 2 versus 14 pts in group 1 (8.3% vs 63%, p < 0.01). The time to neutrophil recovery did not differed significatively in both groups (6.2 days in group 1 vs 5 days in group 2).
Conclusions: Filgastrim reduces the incidence of mild mucositis in pts with non-Hodgkin lymphoma treated with moderately intense chemotherapy protocols and this effect is not mediated by neutrophil recovery, suggesting a direct local effect in the digestive mucous.

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HEPATITIS B VACCINE: IMMUNE RESPONSES IN IMMUNOCOMPROMISED CHILDREN WITH CANCER

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Hepatitis B virus infection (HBV) has been a major complication in immunocompromised children with cancer. Unlike healthy adults, a high proportion of them become chronic carriers with the serious consequences of chronic active hepatitis, cirrhosis and hepatocellular carcinoma. In preparation of the vaccination trial at our Department, we showed that 19% of our patients (pts) were positive for anti-HBc and anti-HBs, whilst 8.2% were chronic HBsAg carriers. The present study was undertaken to elucidate the antibody response of our pts to hepatitis B vaccination, which has been enhanced by increasing the number of doses. **Pts and methods.** In a prospective controlled trial we enrolled 140 pts (80 boys, 60 girls) with haematologic malignancies and solid tumors, 2-18 yrs of age. According to the status of therapy we devided these pts into two groups: **Group A:** 64 pts in complete remission and off therapy, **Group B:** 76 pts under chemotherapy. Eligibility requirements in the study included negative serologic studies for all markers for HBV, HCV and HIV. Recombinant HB vaccine (Engerix 20mcg/dose) was administered in 4 doses at 0, 1, 2 and 6 months in the deltoid region I.M. Blood samples were obtained once every month after each dose. All serum samples were tested for HBsAg, anti-Hbc, anti-HBs (IMX-Abbott Lab.). Protective anti-HBs titres were considered those > 10mU/ml. **Results**

	Anti-HBs>10mIU/ml		
Months	Group A (n=64)	Group B (n=76)	Total (n=140)
1	8 (12.5%)	4 (5.2%)	12 (8.5%)
2	11 (17.1%)	9 (11.8%)	20 (14.2%)
3	44 (68.7%)	16 (21.0%)	60 (42.8%)
7	56 (87.5%)	24 (31.5%)	80 (57.1%)

One month after the fourth dose 80/140 pts (57.1%) had seroconverted. In Group A (87.5%) had protective anti-HBs levels while those in Group B (31.5%). The geometric mean titre was higher in Group A than in Group B. **Conclusions.** Our results support the contention, that immune deficiency of children with cancer under chemotherapy, is responsible for the limited seroconversion to HB vaccination. About 30% of these children, however, have the potential for a satisfactory immune response. Therefore we recommend HB vaccination in paediatric pts after diagnosis of malignancy whenever a high prevalence of HBV infection exists.

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INFECTION RATES ASSOCIATED WITH INDWELLING CENTRAL VENOUS ACCESS DEVICES (ICAD) IN CHILDREN; COMPARISON BETWEEN EXTERNCIZED CATHETERS AND IMPLANTED PORT

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The aim of this study was to assess the comparative risks of infection associated with indwelling and with external central venous access devices (ICADs) in pediatric oncology patients.
Patients and Methods: We retrospectively examined the records of 69 pediatric oncology patients who underwent 81 ICAD placements over 5 years. There were 51 external and 30 internal placements with a median follow-up of 232 days.
Results: The risk of infection when using internal ports was 0.06 per 1000 days of catheter use while that of external catheters was 0.17 per 100 days. The difference was most notable in younger children. There was a subjective impression of better psychological adjustment to the internal port.
Conclusion: On the basis of this small study of infectious complications arising from ICAD use, internal ports seem advantageous.

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COMPLICATIONS ASSOCIATED WITH INDWELLING CENTRAL VENOUS CATHETERS

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The use of implanted central venous catheters (CVC) has increased significantly in paediatric oncology because they provide a reliable and safe venous access for therapeutic purposes and supportive therapy avoiding the danger of tissue infiltration caused by corrosive drugs. Between 1991-1996 we have treated 46 patients with combined chemotherapy through CVC (Cuff-Cath, Viggo Spectramed). The aim of our retrospective study was to determine the frequency of acute and chronic complications associated with indwelling catheters. The following data were evaluated in each patient: date of catheter insertion, acute complications associated with catheter insertion (pneumothorax, bleeding), date of catheter removal and chronic complications. Chronic complications were classified as infection or thrombosis. 49 catheters were implanted in 46 patients without acute complications. The median duration of catheters in place was 6.5 months (range:1-13 months). 24 patients experienced some form of chronic complication of whom 14 developed infection and 10 suffered thrombosis. 12 of the 14 infections were local infiltration and 2 patients had symptoms of systemic infection. The most frequently *observed infectious* organisms were Staphylococcus aureus and Staphylococcus epidermidis. The patients having systemic infections needed the catheter removed. In each case of thrombotic manifestation thrombosis had occurred in the catheter, but their occlosure was readily reversed by the local administration of streptokinase. 12 patients had other local complications at the catheter site including erythema, catheter breakage, catheter dislocation and displacement. In conclusion the indwelling catheter has established an important role in the treatment of children with cancer. In our experience the risks of complication caused by CVC were outweighed by the grate advantages of its application.

P-78

RECOMBINANT HUMAN ERYTHROPOIETIN THERAPY FOR PEDIATRIC ANEMIC CANCER PATIENTS RECEIVING CHEMOTHERAPY

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BACKGROUND. Cancer is frequently associated with significant anemia and may be related to inadecuated erythropoietin (EPO) production. **OBJECTIVES.** To assess the efficacy and safety of r-HuEPO in increasing hemoglobin (Hb) level and reducing the need of blood transfusion in children with solid malignant tumors. **PATIENTS AND METHODS.** An open, pilot trial was performed, including 25 patients with solid malignant tumors receiving cyclic combination chemotherapy. Patients with renal insufficiency, or anemia caused by bleeding or hemolysis, and patients with iron deficiency or megaloblastic anemia were not entered in the study. r-HuEPO (epoetin-B, Boehringer Mannheim, Barcelona, Spain) was administered subcutaneously, 150 IU/Kg x 5/wk for 12 weeks. Response was defined as the achievement of a Hb increase of at least 2 gr/dL. Patients were compared to 25 historical controls matched for age, sex and tumor type. **RESULTS.** Baseline parameters were similar ($p>0,05$) in both groups. Mean age $\pm$ SD was 12,6 $\pm$ 2,7 in EPO group and 11,8 $\pm$ 3,6 in controls. Mean Hb increase was greater in EPO group than in controls (2,6 vs. 0,1 gr/dL; $p<0,001$) and the mean units of blood transfused were lower in treated patients (0,35 vs. 3,56; $p<0,001$). Response to r-HUEPO was achieved in 18 patients (72%). No serious adverse effect of r-HuEPO was observed. **CONCLUSIONS.** r-HuEPO treatment represents a safe and effective means to increase Hb level and reduce the need for blood transfusions in pediatric cancer patients receiving chemotherapy.

P-79

NUTRITIONAL STATUS OF CHILDREN AND ADOLESCENTS WITH OSTEOGENIC SARCOMA: PRELIMINARY RESULTS

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PURPOSE: To study retrospectively the nutritional status at diagnosis influencing prognostics factors in children and adolescents suffering from osteogenic sarcoma. **PATIENTS AND METHODS**: From October/1991 till December/1995, 99 patients (pts), 54 boys and 45 girls, aged 60 to 264 months (median 168) with osteogenic sarcoma were evaluated according to their nutritional status. It was used antropometric parameters (A.P.) (weight, height and age) collected at diagnosis. Weight for height of each patient was compared with a national standart (Marcondes), Metropolitan Insurance Life and National Center of Health Statistics (NCHS) in agreement with the age and sex. Pts were evaluated according to kind of sugery (K.S.) amputation-Ap or conservative-Co; initial tumor size (I.T.S.) >12cm or <12cm and grade of necrosis (G.N.) I, II, III, IV or no grade (N.G.). **RESULTS**: A.P. were normal in 54 pts (54,54%), 34 (34,34%) had malnutrition and 11 (11,11%) were obeses. Related with 64 evaluable pts: 10 (15,62%) were obeses, 36 (56,25%) normals and 18 (28,12%) undernourished. **Nutritional status x I.T.S.**: undernourished - <12 cm - 8pts (44,44%), >12 cm - 10 (55,55%); normals - <12 cm - 24 pts (52,17%), > 12 cm - 22 (47,82%). **Nutritional status X G.N.**: undernourished I - 03 pts (16,66%), II - 04 (22,22%), III - 05 (27,77%), IV - 01 (5,55%), N.G. - 05 (27,77%); normals - I - 14 pts (30,43%), II - 07 (15,21%), III - 11 (23,91%), IV - 04 (8,69%), N.G. - 10 (21,73%). **Nutritional status x K.S.**: undernourished - Ap - 08 pts (44,44%), Co - 10 (55,55%); normals - Ap - 13 pts (28,26%), Co - 33 (71,73%). The authors found no association between nutritional status and prognostic factors (p>0,001). **CONCLUSION**: Although 50% of the patients, among us, have late diagnosis reflected by the tumors size, ≥ 12 cm, after presurgery chemo, it was impossible to demonstrated the relation between nutritional status, diagnosis and prognostics factors (ITS, GN, KS), probably because it is a solid tumor with is slowly consuptive and because of the low number of pts, as it was mencioned in leukemia studies. This study goes on, adding methods of the determination of triceps skinfold thickness.

P-80

ALTERNATIVE THERAPY (AT) USE IN PEDIATRIC ONCOLOGY PATIENTS

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Background: Interest in AT for cancer treatment is on the rise. Studies indicate that 10% to 50% of cancer patients use AT. The prevalence of AT use by pediatric cancer patients is not well defined. We conducted a pilot survey to determine AT use in a pediatric oncology population of a large, tertiary care referral center. We examined 1) sociodemographic factors and diagnosis of users, 2) sources and costs of treatments, 3) patient perception of therapy effectiveness, and 4) notification of physician by family of AT use.
Methods: A questionnaire consisting of 20 multiple choice and 13 open-ended questions was sent to 145 pediatric patients undergoing conventional chemotherapy for cancer. Forty-nine (34%) completed questionnaires were received. Due to the anonymity of the study no further follow-up was attempted. The 49 patients are the subject of this report.
Results: Fifteen (30%) respondents used one (n=4) or more (n=11) alternative therapies. Vitamin and mineral supplements were used most frequently (n=8) with diet alternatives second (n=6). Of patients using alternative therapies, 80% had at least one family member using alternative therapy. Only 3 of 34 (8%) non-users reported family member use of complementary therapy. Socioeconomic characteristics of AT users were similar to non-users. AT was provided by health food stores (53%), chiropractors (27%) and massage therapists (20%). One half of families obtained therapies from more than one source. Expenditures per month were ²$50 (60%), $51-$100 (20%), >$100 (10%). AT was perceived as moderately to extremely helpful by 80% of users and >90% intend to continue AT beyond completion of conventional treatment. The majority of AT users (93%) had discussed this with their physician.
Conclusion: A significant number of pediatric oncology patients use one or more AT. AT use by family members is predictive of use by the patient and may be a useful screening question for identifying patients likely to use AT.

P-81

EVALUATION OF THE EFFECTIVENESS AND COMPLICATIONS RELATED TO THE USE OF AMPHOTERICIN B IN CHILDREN AND ADOLESCENTS WITH CANCER

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Amphotericin B (Amph. B) is currently a widely used drug for the treatment of fungal infections in oncology patients (pts). Fungal infections in oncology pts are an important cause of mortality due to the intensive use of chemotherapy and broad spectrum antibiotics. In order to evaluate the efficacy and complications of Amph. B, the authors studied 55 pts during the period of January 1994 to December 1995. Their mean age was 7,4 years (range 1-18 y) and their distribution was the following: leukemia (20), lymphoma (11) and solid tumors (24). Indications of Amph. B were: a) empirical (0,5 mg 1 kg day) in 27 pts with fever (F+) and granulocytopenia (G+) during the 5th - 7th day of treatment with broad spectrum antibiotics; b) therapeutic (1 mg 1 kg day) in 23 pts with F+ and G+ with clinically documented infection: mucositis (10), esophagitis (8), pneumonitis (3), granuloma (2); c) therapeutic, in 5 pts with F+ but non-granulocytopenic (G-) with well documentated fungal infection. Amph. B was administrated I.V. in a 3 hours infusion with pre-medication schedule: dipyrone, hydrocortisone and dimenhydrinate. Efficacy was demonstrated when fever ceased in 48 hours in 28/50 pts F+G+ (56%) and in 72 hours in 34/50 pts F+G+ (68%). In the 48 hours evaluation, there was associated elevation of granulocytes in 8/50 episodes (16%) and the fever ceased without granulocytes recovery in 20/50 (40%). All pts presented granulocytes recovery (median time 10 days: 5-31 days) and fever ceased in a mean period of 2,9 days (1-14 days). No deaths due to uncontrolled fungal infection occurred. Adverse reactions occurred in 28/55 pts (50%); 24/28 (85,7%) were hypokalemia (reverted after correction), transient renal impairment in 3/28 and generalized exantema in 1/28. Amph. B proved to be an efficacious drug for the treatment of oncology pts with F+ G+ with low grade and reversible toxicities. Dose and mode of administration were satisfactory concerning empirical and therapeutic use, however, we recommend potassium supplementation.

P-82

COMPLICATIONS RELATED TO SUBCUTANEOUSLY IMPLANTED PORTS (IP) IN A PEDIATRIC ONCOLOGY UNIT

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Central venous access is important for the management of patients undergoing intensive chemotherapy.The use of subcutaneously IP allows safe administration of antineoplastic drugs and parenteral support therapy. This study analysed the long-term function and complications related to the use of IP in 112 consecutive children (118 catheters) with malignant disease treated with intensive chemotherapy regimens in a paediatric oncology unit from 1989 to 1995. There were 66 boys and 46 girls age ranged 3 months to 21 years (median 7 years). The diagnosis were 33 leukaemia/lymphoma and 79 solid tumours The period of use of IP ranged from 9 days to 40 months (median 10,5 months). Removal of IP occurred in 16/118 (13,5 %) catheters: related to infection in 8/118 (6,7 %), obstruction in 6/118 (5,1 %), extravasation in 1/118 (0,85%) and dislodgement in 1/118 (0.85%). Removal of the IP associated with fungal or repeated bacterial'IP colonisation was more frequent than in previous reports, including an study from our unit suggesting that IP were more appropriated than externalised catheters in population with limited resources to perform frequent catheter flushing. *Staphylococcus aureus* was the commonest isolated organism, followed by *Candida sp.* The safe and prompt venous access provided by IP are of extreme value for these children; however we have to consider the high rate of complications. The decision as to which of the devices is most appropriated in an individual patient depends on the type of therapy being administered and the patient's and/or physician's preference. The patient's social/economic background may be an additional factor to be placed in perspective.

P-83

UROGENITAL PSEUDOMONAS INFECTIONS IN IMMUNOCOMPROMISED PATIENTS UNDER CHEMOTHERAPY

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The recent utilization of more intensive chemotherapy regimens has caused an alteration in the epidemiology of bacteriemia in children and adolescents with cancer. This alteration were noted as an increase in frequency of gram (-) bacteriemias especially Pseudomonas Aureginosa by many authors. As an uncommon but serious presentation we observed 5 cases of urogenital Pseudomonas Aureginosa infections in our clinic parallel to the increase in Pseudomonas Aureginosa bacteriemias, in last two years. Three of them were ALL, having their induction chemotherapies for at least three weeks, one of them was Burkitt lymphoma and also having COMP chemotherapy protocol for three weeks, the remaining one was AML having MACE protocol as consolidation chemotherapy. All the patients were neutropenic when they were affected. They had their infections around vulva in three of them and around glanspenis, anus in the others, just one of them also had positive hemoculture. The infections were treated in all for at least three weeks with various combinations of imipenem, ciprofloxacin, piperacillin together with local antiseptic measures. Pentaglobulin was used in three of them according to their clinical status. Empirical antifungal therapy and intravenous immunglobulin 0,2 gr/kg weekly were also given as in all neutropenic patients in our clinic. Surgical debridment was used in one patient without any complication. As a conclusion when the patients are neutropenic even a serious Pseudomonas infection in the urogenital region might be treated succesfully if a rapid approach and a careful follow up is maitained.

P-84

PRIMARY BONE TUMOURS IN CHILDREN: EPIDEMIOLOGICAL ASPECTS

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Epidemiological,experimental and clinical data indicate that some environmental agents,like cadmiu,lead zinc and radium are osteotoxins in man and other species. Aware about our important chemical pollution,demonstrated by high levels of cadmiu,lead and zinc in drinking water and some biofites we aimed at evaluating the epidemiological situation of primary bone cancers in children in our county. Our data derivated from the anathomopathological registry and from the records of the children diagnosed with primary malignant bone tumors in the period of 1985-1995. Bone tumors in children represented 8,8% of all childhood malignancies,and a proportion of 31% of the bone malignancies in adult. The most affected group of age was 10-16 year,and the predominant site (30,1%) was the femur. The histopathological review showed: osteosarcoma-56%,primary lymphoma of bone - 12,9%, osteosarcoma associated with retinoblastoma -4,3%, chondrosarcoma -4,3%, chondro-mixosarcoma -4,3%, rhabdomyosarcoma(direct extension) -4,3%,Ewing's sarcoma -4,3%, condro-mixoid fibrosarcoma -4,3%, neuroblastoma -4,3%. The annual incidence of bone tumours in children was situated in our county between 0,072/100.000/year — 0,28/100.000/year suggesting that the exposure to the above mentioned environmental agents doesn't act as a risk factor for this group of malignancies,at least during the childhood.

P-85

MONITORING THE EFFECT OF PREOPERATIVE CHEMOTHERAPY IN BONE TUMOURS WITH DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING: RESULTS OF A 5-YEAR PROSPECTIVE STUDY

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PURPOSE : To assess the value of dynamic contrast-enhanced magnetic resonance imaging (MRI) for monitoring the effect of preoperative chemotherapy in malignant bone tumours.

METHODS : Since 1990, 20 patients presenting with a malignant bone tumour (osteosarcoma, Ewing's sarcoma and bone lymphoma) were examined with dynamic contrast-enhanced MRI before biopsy, during and at the end of preoperative chemotherapy. Physiologic MR images, displaying the enhancement rate during the first-pass of an MR contrast agent through the tumour, were correlated point-to-point with histopathologic findings of the biopsy and the resected specimen.

RESULTS : The physiologic "first-pass" images visualized tumour microvascularization, perfusion and capillary permeability with a spatial resolution down to 2 mm^2. Highly vascularized and perfused, malignant areas were displayed light grey to white, whereas slowly perfused areas (e.g. heavily damaged tumour tissue after preoperative chemotherapy) were displayed dark grey to black. There was a good correlation between the grey scale of these images and tumour vascularization and perfusion ($r = 0.93$; $p < 0.05$). As the grey scale of these images corresponded to the enhancement rate, they allowed to indicate the best site for biopsy by visualizing the areas with the most actively perfused tumour tissue. Monitoring of preoperative chemotherapy was possible by visualization of a decrease or increase of areas with viable tumour. Responders could be differentiated from non-responders by direct estimation of the degree of tumour necrosis and by detection of residual viable tumour nests down to 2 mm^2.

CONCLUSIONS : Physiologic, contrast-enhanced MR imaging allows to indicate the best site for biopsy and to monitor the effect of preoperative chemotherapy, by detection of viable tumour and estimation of tumour necrosis.

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RESPONSE OF OSTEOSARCOMA TO PRE-OPERATIVE CHEMOTHERAPY : ACCURACY OF DYNAMIC-ENHANCED MRI AND SKELETAL ANGIOSCINTIGRAPHY.

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Abstract:
Purpose : To study the usefulness of Dynamic-enhanced MRI and skeletal angioscintigraphy for prediction of good vs bad responders to neo-adjuvant chemotherapy for osteosarcoma.
Material and methods : 15 consecutive patients refered in our institution from 1992 to 1995 with resecable osteosarcoma were prospectively studied with Dynamic-enhanced MRI and skeletal angioscintigraphy. Patients were evaluated before neo-adjuvant chemotherapy, after 2 courses and finally after 4 courses just before the excisional surgery. Skeletal angioscintigraphy was labelled with Tc^{99m}. Dynamic uptake of radionuclide was studied every 2,5 seconds from injection during 3 minutes. Gadolinium enhancement on dynamic-MRI was studied every 30 sec during 3 minutes after injection. Angioscintigraphy time-uptake intensity and MRI time-signal intensity curves were calculated. The initial part of the slopes were compared for each imaging tool before treatment, at midtime of chemotherapy and before surgery. These data were compared to the post therapeutic histopathological findings according to the Huvos-Rosen grading.
Results :
- At initial evaluation, the slopes of time-intensity curves on dynamic MRI and angioscintigraphy correlated well together.
- After 2 courses of chemotherapy, both imaging tools failed predicting with accuracy good vs bad responders.
- After 4 courses, imaging results correlated better with findings of Huvos-Rosen grading :

Results after 4 courses of CT		PPV	NPV	SENSIT.	SPECIF.	TOTAL ACCURACY
Huvos Good Responders	Dynamic-MRI	86 %	100 %	71 %	100 %	85 %
	Angio-scinti.	83 %	83 %	83 %	83 %	85 %
Huvos Bad Responders	Dynamic-MRI	100 %	75 %	100 %	71 %	85 %
	Angio-scinti.	83 %	83 %	83 %	83 %	83 %

Conclusion :
- Early Tc^{99m} uptakes and early Gadolinium enhancements fail to predict the final tumor response at the midtime of neo-adjuvant chemotherapy. These results differ from previous published data.
- On the other hand, accurate prediction of response is equally possible after 4 courses of chemotherapy both with angioscintigraphy or dynamic MRI in osteosarcoma. These results could be usefull for management of non surgical osteosarcoma. Thus, these imaging tools could be used in the future for therapeutic evaluation if neo-adjuvant chemotherapy protocols are prolonged or changed in case of non response after 4 courses.

P-87

MRI - FOLLOW - UP IN COMBINED CHEMO - AND RADIOTHERAPEUTIC TREATMENT OF EWING TUMORS

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Purpose: MRI in Ewing tumors (ET) has been demonstrated as an effective method to monitor the results of chemotherapy, radiation or surgical therapy. We investigated signal intensity characteristics of ET on consecutive MRI follow-ups in patients (pts) under combined chemo- and radiotherapy where the primary tumors were not or delayed resected.
Material and Methods: Up to now 11 pts (7m, 4f; age 8 mo to 22y11mo) underwent serial MRI (0.2-1.5T; T_1+T_2-wSE-Images+STIR+T_1-wGd-DTPA enhanced SE Images; average 5 MRI in the follow up). Chemotherapy was applied according to CESS 86 or EICESS 92 and accompanied by local radiation therapy (42-55Gy). Signal intensities (SI) of soft tissue and marrow components of the tumors were compared to muscle and bone marrow.
Results: At presentation the soft tissue component showed high SI on T_2-wI and low or iso SI on T_1-wI with enhancement after contrast. The bone marrow component had high SI on T_2-wI and low SI on T_1-wI. During treatment early loss of soft tissue component was accompanied by a decrease in SI on T_1-wI, by an increase in SI on T_2-wI and lack of enhancement after Gd-DTPA of the bone marrow tumor component. At delayed surgery or extended surgical biopsy grades of regression were 1 in 3 pts and 3 in one pt according to Salzer-Kuntschik.
Conclusion: MRI seems to be unreliable for exclusion of active disease in ET after combined chemo-and radiotherapy. However, a characteristic pattern of change in SI and contrast enhancement is qualitative evidence of therapeutic effects.

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STAGING OF OSTEOSARCOMA ON MAGNETIC RESONANCE IMAGING (MRI) : CORRELATION OF MRI WITH PATHOLOGICAL MACROSLIDES.

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Between October 1993 and February 1995, five patients with osteosarcoma of a limb were treated at this institution. There were two females and three males with femoral (n=3) and tibial (n=2) osteosarcoma.
Following chemotherapy, MRI was performed on a 1.5 Tesla sigma GE machine using T1 weighted and fast multiplanar inversion recovery sequences.
The intramedullary and periosteal extent of tumour were measured using electronic calipers. Patients then underwent surgical resection of their residual tumours and macroslide preparations of the excised specimens were obtained.
We compared intramedullary and periosteal extent of tumour as measured on both sequences, with those obtained from the excised specimen.
The results showed that intramedullary extent of tumour could be accurately predicted on T1 weighted images to within 1 - 3 mm, however, periosteal extent was overestimated on both T1 weighted and fast multiplanar stir images by between 3 and 73 mm.
This limited study has shown that T1 weighted images on this machine are highly accurate in determining intramedullary extent of osteosarcoma, as has been shown by others (J. Bone Joint Surg. 1986, 6, 68A).
However, our ability to help surgeons decide levels of amputation by estimation of periosteal extent of tumour remains poor (Radiology, 1988, 167, 765-767). Despite our use of latest technology, our attempts are hindered by our inability to distinguish tumour from associated oedema.

P-89

THE ROLE OF FNAC IN THE DIAGNOSIS AND MANAGEMENT OF CHILDREN WITH BONE LESIONS

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FNAC of primary and metastatic bone lesions was performed on 70 children (age: 5 month-16 years) reffered to our pediatric oncology clinic. FNAC was performed under X-ray guidance in 15 cases. The others were directly approached by the cytologist. Adequate material for cytologic evaluation was obtained in 65 of the 70 patients. Of the 65 aspirates, 36 were diagnosed as malignant and 28 as benign; the remaining case was inconclusive. The diagnosis included: osteosarcoma (n=20), Ewing's sarcoma (n=2), non Hodgkin lymphoma (n=2), cysts (n=6),eosinophilic granuloma (n=4), benign lesion NOS (n=3). osteoblastoma (n=1), chondroblastoma (n=1), osteochondroma (n=1) and inflammatory lesion (n=6). FNAC diagnosis was confirmed either by histology (60 cases) or by clinical evaluation (in metastatic cases). We concluded: **1** - FNAC is a useful method in the diagnosis of bone lesions when used in correlation with clinical and radiological data. **2** - The differential diagnosis between benign and malignant tumors was possible in all but one case (98,6 %). **3** - A specific diagnosis was always rendered in malignant tumors, but is very difficult in benign lesions.

P-90

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) IN DIAGNOSIS OF OSTEOSARCOMA AND EWING`S SARCOMA

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Osteosarcoma is the most common malignancy of bone in children with a slight predominance in boys. Second in incidence only to osteosarcoma among the primary malignant childhood tumors of bone Ewing`s sarcoma has a predilection for children in their early to mid-adolescent years. The object of this study is to determine the diagnostic value of FNAC in bone malignant tumours, as this method can be used before treatment selection. FNA`s were performed in 43 patients in the period of 199o-1995. Of these 43 patients 27 were boys and 16 were girls, their ages ranging between 1 to 16 years. In the group of 43 patients; 2o cases were osteosarcoma and in 15 patiensts FNAC diagnoses were performed; 23 patients were Ewing`s sarcoma and FNAC diagnoses in 2o patients. Thus in 15 cases of osteosarcoma and in 2o cases of Ewing`s sarcoma, the cytologic and histopathologic results were comparable, with no false positives and false negatives. In this article we discuss the correlation between FNAC and histopathologic findings, the distribution of such tumours in realtion to age, sex, initial localisation. Furthermore, the advantages of FNAC are presented in early detection of metastases, before and during therapic procedure. In conclusion it can be stated that FNAC has a significant role in the diagnosis of osteosarcoma and Ewing`s sarcoma, and that possibly FNAC before treatment is a successful technique in the diagnosis of osteosarcoma, Ewing`s sarcoma, and also during a early detection of metastases.

P-91

CORRELATION AMONG OVER-EXPRESSION OF P-53 PROTEIN AND DEGREE OF TUMOR NECROSIS IN OSTEOSARCOMA

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Degree of necrosis of 32 patients with osteosarcoma after chemotherapy, was analyzed, 1 case presented less than 25%, 10 cases between 25 - 89% and 17 cases 90-99% and 4 cases 100% to the degree of tumor necrosis
The nuclear protein P-53 is coded by the P-53 supressor gene localized on the short arm of human chromossome 17 acts as a supressor of cell division. The P-53 tumor supressor gene is mutated in 60% of human tumours. The antibody P-53 protein then gives preferential labelling of malignant cells in which the mutant P-53 protein accumulates. Staining is predominantly nuclear in malignant cells.
Results showed over-expression of P-53 protein in 5 from 11 cases (45%) with degree of necrosis between 0-89% and 5 from 21 cases (23%) between 90-99%.
In 16 patients (76%) with good response to chemotherapy (more than 90% to degree of tumor necrosis) didn't showed over-expression of P-53 protein.
This study sugest that over-expression of P-53 protein is correlated with poor response to chemotherapy. Analysis of surgical specimens at diagnosis is necessary to confirm these findings.

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Title (CAPITAL LETTERS): Comparison of the histopatological patterns of central osteogenic sarcomas observed from the initial biopsy, through tumorectomy to pulmonal metastases.

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Abstract

The histology of the pulmonal metastases in osteogenic sarcoma was analized in the aim to found the dependance on the primary focus. 28 cases of pulmonal surgery performed because of metastases were chosen for analysis. The age range was 3-18, the average age 12,5 yrs. The initial biopsy and the tumorectomy were histologically examined according to two generally used classiffications i. e. the gradual , /I-III/ and the structural one. The percentage of the tumours necrosis was established after the performance of the histological map. All cases underwent the chemiotherapy just after the initial biopsy.

The obtained results were: the stability of the histopatological features from initial biopsy, through surgery to metastases in 8 cases; 20 times changes of the histology were established. In 5 cases the following dedifferentiation,in 6 the increase of differentiation of metastases was stated;in 9 the dominant histological patterns varied without change of degree of malignancy. We do not find any correlation among the degree of the tumours necrosis, the position in the gradual and structural classification of the primary focus and the histological patterns of the metastases. It was evident that the generally most numerous cases were grade II, with osteoblastic histology and tumours necrosis between 50-80%.

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BONE LESION OF CAT-SCRATCH DISEASE (CSD)

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Objective:

An unusual case of CSD with costal location is reported to emphasize potentially misleading presentation and the need of a prompt recognition to avoid invasive diagnosis method.

Case History:

A ten-year-old previously healthy girl was referred to the hospital for a recurrent high fever and abdominal pain. On admission, her examination was unremarkable (no adenopathy; no rash; neck, lung, heart: normal). Laboratory data were normal (except elevated fibrinogen, ESR and C-reactive protein). Antibiotics were prescribed.

On fourth hospital day, the patient complained of an acute thoracic right-sided pain. Physical examination, chest and abdominal X-rays remained normal. A CT scan confirmed absence of intrathoracic lesion. A radionucleide scan showed an increased uptake at the 11th right rib. An RMI study revealed a rib fracture with soft tissue infiltration and demonstrated multiples hepatic nodules which appeared hypoechoic on abdominal ultrasonogram.

Considering acute clinical onset, absence of trauma, negativity of blood and stool cultures, and negative serologic studies, a primitive bone tumor with metastatic spread was suspected. An excision-biopsy was performed.

Histopathology of the surgical specimen showed chronic granulomatous osteomyelitis. These findings with an history of contact with a pet cat, occasional scratch cutaneous lesions and a low positive skin test for CSD (sero test was not available) led to the diagnosis of CSD.

Follow-up showed complete resolution of hepatic lesions within 7 months and calcification of the costal site.

Conclusion:

CSD should be considered in the diagnosis of bone lesions ocurring with fever of unknown origin in children and young adults without lymphadenitis.

P-94

SOME IMMUNOPATHOLOGICAL FEATURES OF EWING'S SARCOMA.

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Flow immunocytometry, immunomorphological study of TILs and tumor cells as well as tumor ploidity, number of cells un G0/G1, S and G2+M phases of cell cycle measurement was performed in 29 Ewing's sarcoma cases. Tumor cells were positive for E2 (CD99), HLA class I and CD71. Different degrees of positivity for the following antigens were noted: CD9, CD107b, CD109, 4F2, L129, L106, F10-3. The possibility of induction of apoptosis during Ewing's sarcoma treatment was stated, expression of APO-1/FAS being absent in untreated tumours. Common leucocyte markers (CD45, CD50) as well as T-/B-/myeloid antigens (CD1, CD2, CD3, CD4, CD5, CD7/CD19, CD22/CD11b, CD14, CD15) were not expressed on Ewing's sarcoma cells. CD38, CD10, CD25, CD26, CD39, HLA-Dr were also absent. Some neuroectodermal antigens (HSAN, GD2, Thy-1) were inconstantly positive. Expression of adhesion and activation antigens was heterogeneous. CD29-negative Ewing's sarcoma variant associated with minor T-cell infiltration/activation was identified. Aneuploid tumors had significantly higher ($p<0,05$) proliferative activity, larger proportion of cells in S-phase and more aggressive coarse.

P-95

COMPLEX CYTOGENETICAL CHANGES INVOLVING THE EWS-ERG FUSION GENE IN A PNET CELLINE

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Ewing's sarcoma and PNET are characterized by translocations between the EWS gene located on chromosome 22q12, and various partner genes including the FLI1 gene (chr. 11q24) and the ERG gene (chr. 21 q22.2), both members of the ETS-family genes.
Cytogenetical studies of the PNET celline CB-AGPN revealed a complex karyotype, with no evidence of involvement of chromosome 11, 21 and 22: 47,X,t(X;8)(p11;q24),+der(8)t(X;8)(p11;q24),der(13)del(13)(q21q32) add(13)(q32),der(16)t(1;16)(q11;q11),add(19)(q13). FISH studies with a cosmid probe for the EWS gene revealed a translocation of this region to the derivative chromosome 13. To further analyse this unusual translocation we used paints for chromosomes 11, 13, 19 and 21, and demonstrated a three way translocation t(13;19;22)(q21;q13;q12). RT-PCR using primers
for the EWS-ERG fusion gene, produced a product with 100% homology of a fusion gene with a junction between exon 7 of EWS and the predicted exon 6 of ERG.
We propose that the original genetical change is a t(21;22)(q22;q12) followed by the involvement of the der(22) in a complex translocation with chromosomes 13 and 19, loss of the der(21) and duplication of the normal 21 homologue.

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INDUCTION OF CD3+/CD56+ KILLER CELLS IN A CHILD WITH PERIPHERAL NEUROECTODERMAL TUMOR BY IMPLANTATION OF AUTOLOGOUS IL-2 TRANSFECTED FIBROBLASTS

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Posttransplantational adjuvant interleukin-2 (IL-2) therapy has previously been shown to improve prognosis of patients with Ewing tumors. This has been attributed to a stimulation of the immune system and its antineoplastic activity, thus eliminating minimal residual disease. In preclinical studies we have shown that transduction of cytokine genes into Ewing tumor cells leads to lymphocyte activation and tumor cell lysis. As the amounts of cytokines produced locally are low, systemic toxicity should not occur. An 11-year old patient suffered from a peripheral neuroectodermal tumor in 5th relapse, refractory to chemotherapy, surgery, radiation and thermochemotherapy. Autologous fibroblasts were transfected with an IL-2 gene expression vector. An 6kb expression plasmid (pRC) was used carrying the entire human IL-2 cDNA, driven by a CMV promoter, and the Neomycin resistance gene. Having established a cell line of the patient's fibroblasts, the cells were transfected using a cationic liposome reagent. 2 - 7 x 10^6 transfected cells were applicated by injection into the tumor under CT-guidance in weekly intervals x 3. No side effects were observed. Peripheral blood samples were drawn in weekly intervals following the injections and analysed by flow cytometry. 7 days after the injection of 7 x 10^6 a significant rise in the population of CD3+/CD56+ cells in peripheral blood was shown. These CD3+/CD56+ cells have previously been described as cytokine induced killer cells. In a biopsy taken from the tumor 21 days after the last injection, also analysed by flow cytometry, an increase in the amount of CD25+/CD3- cells in the tumor was demonstrated. These results indicate an in vivo immunostimulatory effect of intratumoral implantation of genetically modified fibroblasts.

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CARBONYL GROUP CONTENT IN PLASMA IN CHILDREN WITH BONE TUMORS AND OTHER CANCERS.

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Toxic oxygen free radicals have been implicated as important pathologic mediators in carcinogenesis. In previous publications we observed that antioxidant barrier can be different in healthy children and children with cancers. We were looking for a stable marker of damaging result of free radicals reactions. Carbonyl group level in proteins have been introduced as an excellent marker of oxidative stress damage. In this study we investigated spectrofotometrically, according to the method of Levine, the concentration of carbonyl groups in serum of 29 children (19 boys and 10 girls) aged from 7 to 16, who had been diagnosed as suffering from cancers (osteosarcoma n=9, sarcoma Ewingi n=4, medulloblastoma n=7, lymphoma malignum n=4, hepatoblastoma n=3, carcinoma embrionale n=2) The control group consisted of 18 age-matched healthy children (13 boys and 5 girls). The results of the study are reported in the table.

PARAMETER	PATIENTS-BONE TUMORS	PATIENTS-BRAIN TUMORS	PATIENTS-OTHER TUMORS	HEALTHY CHILDREN
	MEAN ± STANDARD DEVIATION			
CARBONYLS [nmol/mg protein]	1.82 ± 1.19* n = 13	1.34 ± 0.52^ n = 7	1.74 ± 0.51# n = 9	0.78 ± 0.15 n = 18

#,*,^- statistical significance - # - $p<0.001$, * - $p<0.005$, ^-$p<0.02$

The content of carbonyl groups in plasma proteins has shown a significant increase in children with tumors. A possible interpretation of this data suggests an inadequate antioxidants protection in children with tumors. The relationship between the oxidative damage and carcinogenesis requires future investigations.

P-98

P53 MUTATIONS IN EWING'S SARCOMAS ARE AN UNCOMMON EVENT

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Aims: To provide further insight into the possible role of childhood malignancy of pathways involving p53, we analyzed the mutation spectrum of the p53 gene and the immunohistochemical p53 expression in a group of Ewing's tumors.
Material and Methods: Formalin-fixed and paraffin-embedded tissue of 14 tumors and, additionally, freshly frozen material of 2 patients was available prior to chemotherapy. Exons 4-9 of the p53 gene were analyzed by the PCR-SSCP technique and direct sequencing on an automated fluorescence sequencer. Immunohistochemical staining (monoclonal antibody DO1, Oncogene Science) was visualized by a standard ABC-method.
Results: The age of the patients ranged between 2 and 24 years. 6 of 14 patients died of disease during an average period of 14 months after first diagnosis. No p53 mutations could be confirmed in the conservative regions of the p53 gene. In accordance with this result, nuclear p53 accumulation could not be demonstrated immunohistochemically.
Conclusions: Compared with other highly malignant mesenchymal pediatric tumors such as osteosarcoma, P53 mutations in Ewing's sarcomas are an extraordinarily rare event. This finding corresponds well with the different histogenesis of Ewing's sarcomas, which are not of mesenchymal but of neuroectodermal origin.

P-99

OVER-EXPRESSION OF p53 GENE PROTEIN AND ITS PROGNOSTIC SIGNIFICANCE IN EWING'S SARCOMA OF BONE

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p53 gene mutation is the most common genetic abnormality associated with malignancy. We used immunohistochemical methods to study p53 gene mutations in patients with Ewing's sarcoma of the bone treated between 1983 and 1993 to determine their prognostic significance.

Biopsy tissues of 52 patients with Ewing's sarcoma of bone were studied. Mean age was 17 years and the minimum follow-up was 30 months. The tumours were located in the extremities and central bones in 35 and 17 patients respectively. Seven patients had metastases at diagnosis. Treatment consisted of chemotherapy, surgery and/or radiotherapy in all the patients.

p53 protein expression was demonstrated in 7 patients (14%). There was no relationship between expression of p53 and tumour stage, site, patients' age and necrosis following chemotherapy (p>0.5). The 5-year relapse-free survival and overall survival in patients without metastases at diagnosis were 66% and 71% repestively in p53 negative patients compared to 20% relapse-free and overall survival in those with p53 expression (p=0.01). The poorer prognosis in p53 protein positive patients was independent of site, local treatment or necrosis of the tumours (p=0.02).

Expression of p53 protein is an independent poor prognostic factor in Ewing's sarcoma of bone.

P-100

UROKINASE IN HUMAN OSTEOSARCOMAS

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Subject of investigation was the role of urokinase (u-PA) regarding invasion and motility of osteosarcoma cell line MNNG/HOS. Additionally, in 30 tumor specimens, immunohistochemical findings were evaluated. For intracellular, extracellular and surface-bound fractions of u-PA, enzyme expression of cells were determined by ELISA. Investigations on function were made in invasion and motility chambers. Activity of u-PA was inhibited by anticatalytic antibodies (1µg/ml, 10µg/ml, 100µg/ml). Immunohistochemical investigations were performed on 30 cryoconservated tumor specimens.
MNNG/HOS shows high u-PA concentrations in intracellular, extracellular and surface-bound fraction. Investigations on function revealed that 8.6% of cells are invasive, 45% surmount the filter obstacle in motility tests. Anticatalytic antibodies reduce significantly invasion and motility. Modified invasion activity is 8.1%, 6.8% and 6,2%, motility is 26.6%, 15.8% and 12.5%, respectively. Immunohistochemically, non-chondroblastic tumors, on the whole, show a strong reaction for u-PA. The antigen is located in both tumor cells and stroma. In comparison with non-chondroblastic tumors, there is a weak up to negative immunoreactivity in chondroblastic osteosarcomas or enclosed chondroblastic tumor areas.
Results obtained in vitro demonstrate that urokinase plays an important role in invasion and motility of osteosarcoma cells. On the one hand, high concentrations of enzyme can be proved in all culture fractions investigated, on the other hand, invasion and motility of cells can be significantly reduced by anticatalytic antibodies. Immunohistochemical findings in osteosarcoma specimens confirm these results. Strong expression of urokinase is observed in non-chondroblastic tumors. Chondroblastic tumors only show negative up to weak immunoreactivity, thus correlating with rarer occurrence of metastases in osteosarcomas of this differentiation.

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LARGE RECONSTRUCTION FOR MALIGNANT BONE TUMOR IN CHILDREN. THE ALTERNATIVE OF VASCULARISED FIBULAR BONE GRAFT.

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Late failures of massive allografts and megaprosthesis are often described in children.
That the reason why we used free vascularized fibular bone grafts as often as possible. This technic allows full activities unless the fibula reached mecanically well adapted width.
During the past 5 years, a vascularized fibular graft was indicated in 13 patients : 4 ewing sarcomas and 9 osteosarcomas.
The site lesions were proximal femur (1 case), distal femur (5 cases), proximal tibia (3 cases), distal tibia (1 case), distal fibula (1 case), iliac bone (1 case), proximal humerus (1 case).
In 4 cases the remaining femoral or tibial epiphysis was less than 3 cm after resection.
In one case the vascularized graft was indicated after prosthesis failure, in two cases after long term cryopreserved allograft reconstruction failure, in the remaining 10 cases the vascularized bone graft reconstruction was primary indicated.
No patient showed infection or local recurrence, but one died of pulmonary metastases at 5 years follow up. The graft fractured at 6 months in one patient with tibial reconstruction and in one patient with femoral reconstruction. In both cases consolidation with a large new bone formation was obtained in 2 months.
Compared with other type of bone reconstruction the free vascularized bone graft garantees permanent stability and good fonctionnal status with no fear or breakage. The main disadvantage is the long period before total weight baring. In conclusion we recommand a vascularized bone graft in all cases where after bone resection a short part of the epiphyse can be preserved and after failure of massive allograft.

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ESTIMATION OF LIMB SALVAGE SURGERY COMPLICATIONS IN CHILDREN'S MALIGNANT BONE TUMORS.

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The analysis of 117 osteosarcoma patients treated between 1985 and 1995 is presented. Surgery was performed in 109 patients, in 58 and 51 children mutilating and salvage operations were done respectively. The following types of surgery were carried out: endoprosthesis implantation(26), bone graft implantation(12), rotation plasty(6), stabilization with metal plate(4), clavicle rotation(2), excision of the tumor without reconstruction(1). Surgery was done after neoadjuvant chemotherapy, when stabilization or regression of the tumor was achieved. Early complications (up to 2 weeks after operation) were found in 7 children. In 6 there were vascular in origin. Late complications (up to 6 months) were detected in 3 patients. There were paresthesias, brake of the metal plate, skin necrosis. Complications 6 months after operation were found in 17 children: brake in the place of bone graft(7), infectious(6), mechanical damage of metal elements(4) used for reconstruction. In 24 out of 51 (47%) surgery was not followed by any kind of complication. Of mentioned 51, 45 are alive, but 10 with detectable lung metastases. In 11 children amputations as a results of complications had to be done. In 4, fistulas are still treated.
CONCLUSIONS
1/In 50% of patients complications in the different periods were noted. 2/Early complications were mostly of vascular origin. 3/Very good and good functional result was achieved in 60% of patients(31pts), satisfactory and bad in respective 8 and 1 patients.

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TREATMENT OF PAEDIATRIC BONE TUMOURS BY LIMB SALVAGE OPERATION

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A retrospective review was performed to study the treatment result of paediatric malignant bone tumours using limb salvage operation. From 1990 to 1995, 11 patients were treated with neoadjuvant chemotherapy followed by excision of tumour, and 10 had allograft reconstruction. The patients were diagnosed at a mean age of 10.9 years (range 7.2 - 13.3), and 7 were prepubertal. 10 patients suffered from osteosarcoma (7 lower femur, 2 upper tibia and 1 upper fibula), 1 patient had Ewing's sarcoma of femur. Four patients were treated according to T10 protocol, whereas the others were treated with combination of high dose methotrexate, adriamycin, cisplatin ± VP-16 and ifosfamide. The degree of tumour necrosis was >90% and 60-90% in 5 and 2 patients respectively. Surgery was followed by post-operative chemotherapy. 2 patients had local relapse followed by pulmonary metastasis at 19 and 14 months, and both died. 1 patient died of adriamycin cardiomyopathy at 10 months. Chemotherapy was completed in 6 of 8 survivors and they remained disease free at a median follow up of 19 months (range 11 to 63). 2 patients, prepubertal at diagnosis, have survived for more than 4 years and had limb length discrepancy of > 10 cm. In conclusion, limb salvage operation with allograft reconstruction is an acceptable option for paediatric malignant bone tumours, but limb lengthening operation will likely be required.

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SURGICAL TREATMENT AND ONCOLOGICAL OUTCOME OF PATHOLOGICAL FRACTURES IN NON-METASTATIC OSTEOSARCOMA

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40 patients with pathological fractures through localised osteosarcoma were studied to determine the outcome of limb salvage surgery. All the patients had adjuvant chemotherapy. Median follow-up was 55 months. The femur was involved in 25 patients (63%), humerus 12 (29%) and tibia in 3 patients (8%). 25 patients had fractures prior to diagnosis and 15 patients developed fractures while on chemotherapy.

Limb salvage was performed in 27 patients and amputations in 13 patients. Local recurrence developed in 15% after limb salvage and none following amputations. Cumulative 5-year survival for all patients was 57% The 5-year survival following limb salvage and amputations were 64% and 47% respectively (p>0.05).

Limb-sparing surgery can be achieved despite pathological fractures in osteosarcoma without compromising survival but the risk of local recurrence is significant.

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PRESERVATIVE SURGICAL TREATMENT OF MALIGNANT BONE TUMORS IN CHILDREN : A STUDY OF 17 PATIENTS

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Formerly amputation and disarticulation were often used in the treatment of primitive malignant bone tumors. The increasing use of reconstruction with prosthectic materials associated with efficient chemotherapy regimens has considerably altered functional and vital prognosis. Indeed the survival rate has increased from 12 to 50 % in children. The surgical removal of epiphysial cartilage when performing an extratumor resection results in limbs of different length. This may be avoided by using a growth prothesis.

Seventeen patients under the age of 15 years and a half (mean age : 10 years. 10 months) were treated by extratumoral resection and reconstruction and have a mean follow up of 3 years. There were 12 osteosarcoma, 3 Ewing's sarcoma, 1 chondrosarcoma and one non hodgkinien lymphoma of bone ; location of tumors were as follows : 8 femurs, 4 tibias, 2 humerus, 3 pelvis.

One patient with femur osteosarcoma was initially metastatic.

Reconstruction was either with growth endoprothesis or with standard endoprothesis twice allogenic bone transplant and twice with autologous bone transplant.

Chemotherapy was used before and after surgical removal in Ewing's sarcoma and osteosarcoma. For the patient with LMNH, surgery was performed at the end of chemotherapy.

The functional results of tumors located in inferior limbs were evaluated using the Muskuloskelelal Tumor Society classification put forwards by Enneking. The functional results were excellent or good in the treatment of the knees and average for pelvis. The shoulders were stable and not painful. Multiple operations were necessarily to maintain the equal length of inferior limbs.

The main complication linked to surgery was paralysis of the poplite sciatic nerve in 3 patients, of which two were regressive. We observed the freeing of the prothesis in 3 cases ; two of these were septic and the other was aseptic.

We had 1 local relapse of an osteosarcoma of tibia for which we had to perform an amputation.

Therefore preservative surgical treatment of primary malignant bone tumors in children remains difficult but enables the patient to recover quickly to satisfactory functional status without reducting survival rate. It is an excellent altervative to amputation.

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Title: "LIMB-SPARING SURGERY FOR BONE SARCOMAS OF THE LOWER EXTREMITY IN CHILDREN (6 - 12 YEARS): THE ROLE OF CUSTOM-MADE EXPANDABLE IMPLANTS: OUR EXPERIENCE WITH 22 CASES DURING THE LAST 7 YEARS."

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Amputation surgery was always considered to be the rule for gaining local control in bone sarcomas of the lower extremity in children under the age of 10 - 12 years. The reason was the huge problem of copping with later growth and limb length discrepancies which cause chronic dangerous repeated morbidities until maturity.

During the last 7 years 22 children (5 - 12 year old) with Osteosarcoma or Ewing's sarcoma of the femur or tibia were treated with a variety of custom-made expandable endoprostheses. A total of 24 different implants were introduced (including revisions). About 12 expansions were already performed. 3 children reached maturity.

The complications, especially the prosthesis related ones and funcional results will be described in details. The rate of limb preservation (until now) is 100% - No limb was yet amputated during all the morbidities.

It seems that the goal of maintaining a limb, its length and its function until maturity in these very young children, is an achievable one but with a high price in terms of morbidity (local surgical and psycosocial).

P-107

OSTEOSARCOMA - A RADIORESISTANT TUMOR? LONG-TERM EVALUATION AFTER MULTIDRUG CHEMOTHERAPY AND DEFINITIVE IRRADIATION

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Purpose: Total resection of the primary is believed to be the single appropriate local treatment for osteosarcoma. However, surgical treatment is sometimes refused by patients (pts) or parents. Surgery may be even unreasonable, e.g. in pts with metastatic disease at diagnosis. Thus, an attempt was made to evaluate the effect of radiotherapy as local treatment in combination with multiagent chemotherapy.
Methods: From 1976 to 1995, nine pts with ostesarcoma underwent definitive radiotherapy to their primary lesion combined with multidrug chemotherapy according to Cooperative Osteosarcoma Studies (COSS) of the German Pediatric Oncology Group. Radiotherapy was applied because 7 pts refused appropriate surgery, one was inoperable and one had multiple progressive lung metastases despite chemotherapy.
Results: After radiotherapy all primaries completely regressed, even in two pts who did not respond to chemotherapy. Later on, both succumbed to progessive lung disease without evidence of local recurrence. The remaining 7 pts, all presenting initially with a localized tumor, are alive and well 1.5, 3, 10, 14, 14, 18 and 19 years after the end of treatment. Shape and function of the affected limb is excellent or good in 6 of the 7 pts although 3 of them suffered a transient, slowly healing pathological fracture some time after treatment.
Conclusion: Total surgical resection as the state of the art for local treatment of osteosarcoma has been established at times before the era of modern polychemotherapy. Chemotherapy is not only effective in controlling metastases but also in killing osteosarcoma cells in the primary. Within this concept, the statement that osteosarcoma is a radioresistant tumor is questionable. The results presented here support the hypothesis that local radiotherapy is a reasonable alternative. We would encourage to test this hypothesis in a controlled clinical trial at least in selected pts, e.g. pts in whom appropriate surgery is refused, incomplete, impossible or futile because of uncontrolled metastases or maybe even in pts responding well to initial chemotherapy.

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RESULTS OF INDIVIDUALIZED HD MTX BASED ON ITS PHARMACOKINETICS APPLIED IN THE TREATMENT OF CHILDREN AND ADOLESCENTS WITH OSTEOSARCOMA

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Individualisation of high doses of methotrexate (HD MTX)modified according to its pharmacokinetics in the treatment of children with osteosarcoma (OS)was undertaken during 1992-95 in the aim to improve the treatment results. Modified Rosen protocol was used with 4 preoperative HD MTX courses (crs)and one of 2 postoperative CHT arms with HD MTX/ADR or CDDP/ADR depending on response to neoadjuvant CHT. Drug doses were tailored according to the age and body surface of patients (pts), then modified to obtain therapeutic MTX concentration (TCmax) 1000µmol/l. The effectiveness of the method and therapy toxicity in WHO scale were assessed.
The 90 crs of HD MTX were administered to 22 pts with OS (aged 6yrs 4mos to 28yrs, median age 14yrs 3mos, 15 males and 7 females)based on 522 measurements of serum MTX concentrations using the method of fluorescence polarisation. HD MTX doses 4,0-16,0g/m^2 (mean 9,7±2,4g/m^2), modified in 24,4% of crs (half of them increased)were applied. Toxicity of III and IV degrees concerned 40 crs in 18 pts (44,4%). Hepatotoxicity prevailed (41,1%)mainly in the form of high transaminases activities, then gastrointestinal disturbances (23,3%)and myelotoxicity (15,3%), all with favorable clinical evolution. There were no toxic deaths. Early favorable responses to induction CHT (80,8%)compared with worse ones were influenced significantly by higher mean number of HD MTX crs (3,9 vs 1,8, p=0,013)and higher mean number of HD MTX crs with TCmax (2,24 vs 0,8, p=0,113). Overall survival in pts without metastases (8/11 - 72,7%, half of alive pts limb-spared), with 19mos mean follow up, is good but must be verified in the next 2-3yrs.
Individualized HD MTX are relatively safe and highly effective and should be included in the OS treatment protocols together with CDDP and ADR.

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PHASE II WINDOW WITH HIGH DOSE IFOSFAMIDE (IFOS) AND MESNA (M) IN NEWLY DIAGNOSED CHILDREN WITH METASTATIC BONE AND SOFT TISSUE SARCOMAS .

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Based on our previous experience utilizing high dose IFOS administered by continuos infusion(CI) in previously treated patients (Epelman S, Proc ASCO 12:1412, 1993), we started this phase II study in newly diagnosed metastatic patients. Protocol used was CI IFOS 3g/m2/day over 5 days and an equivalent dose of M during same period. Courses were repeated every 3 weeks for 2 cycles. Concurrent treatment consisted of IV fluid (2l/m2/day of 5% dextrose in 0.25 N Saline + KCl + Ca-gluconate). To date, 35 patients have entered that protocol: 17 osteosarcomas, 7 rhabdomyosarcomas, 5 Ewing's sarcomas and 6 soft tissue sarcomas (non-rhabdomyosarcoma). Age ranged from 2-19 years (median 13 years). All patients had measurable disease. Response was evaluated after delivery of 2 cycles. Six patients achieved complete response, 18 partial response (CR+PR=68%) . Toxicity was tolerable and no death during that period. Major toxicity was pancytopenia with neutropenia (62%), anemia (28%) and plaquetopenia (23%).Two developed severe infection (5.7%). G-CSF was utilized in 12 patients (34%)cycles. Grade 2 neurotoxicity occurred in 3 patients (8.6%). Grade II haemorrhagic cystitis occurred in 1 patients. In summary: This is an encouraging and effective protocol with 68% complete and partial response rate. IT is early to evaluate whether this improved response rate will result in superior survival for those children with newly diagnosed high risk tumors. Due to low toxicity, we recommend addition of other drugs to improve response rate in further studies.

P-110

OSAD93: A MULTICENTRIC PILOT STUDY OF HIGH DOSE IFOSFAMIDE AND CDDP IN PATIENTS (PTS)>16 YEARS OLD WITH NON METASTATIC OSTEOSARCOMA.

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Based on results with the HELP regimen in adults (Proc. ASCO 1993, abstr. 1646), a pilot study for osteosarcoma of pts>16 years was initiated in 1993. Four preoperative courses of SHOC (Ifosfamide 3g/m2 d1 to d3, CDDP:100mg/m2 d4) were given, followed by local treatment. Post operative chemotherapy was: 1) 3 courses of SHOC in pts with ≤ 10% viable tumor cells, 2) 3 courses of HOCA (Adriblastin:60mg/m2, d1 to d2; Ifosfamide:3g/m2 d1 to d2, CDDP: 100mg/m2 d3) in pts with >10% of viable tumor cells.
26 pts were included, of which 21 are evaluable at 02/96 (4F/ 17M, median age: 23 (range:17-62). Tumor sites were: femur (9), tibia (3), humerus (2), pelvis (2), other flat bones (4), ulna (1). The toxicity of SHOC was evaluated in 74 courses. Grade 3 and 4 neutropenia, and febrile neutropenia occured after 46%, 26% and 16% of courses respectively; grade 3 and 4 thrombopenia in 11% and 8% of courses respectively; grade 3-4 vomiting occured after 25% of courses. Other grade 4 toxicities occured in less than 6% of the pts. With 15 months median follow-up, overall and progression-free survival at 1 year are 80% both. Histological response will be evaluated in 08/96 and presented.

P-111

ESTIMATION OF CHEMOTHERAPY REACTION ON INITIAL CHEMOTHERAPY IN RELATION TO HISTOLOGICAL EXAMINATION.

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Analysis of the results in 77 osteosarcoma patients treated between 1985 and 1994 is presented. Preoperative reaction of the tumor on chemotherapy used, was estimated by clinical and radiological methods in relation to histological examination.
Regression and/or stabilisation of neoplastic process was observed in 53 children. Stabilisation of the disease was noted in 31 cases. In 24 out of 77(30%) progression occured irrespective of neoadjuvant chemotherapy.
The mean necrosis rate of about 45% (10%-80%) was found in patients who developped progression. In the group with stabilisation and/or regression this mean rate was 67% (30%-100%). The detailed analysis revealed that such estimation is not in concord with clinical course and final result. It seems that necrosis rate can be used only as guide to estimation of the tumor reaction on chemotherapy used.
The different protocols did not influence the necrosis rates.
The final histologic diagnosis was the same as obtained from biopsy specimens, but in 9 cases the histologic subtypes had to be changed.
The clinical and radiological estimations were similar in 56 cases(73%), in 9 regressions, 31 stabilisations, 16 progressions.

P-112

ASSESSMENT OF ACUTE TOXICITY FOLLOWING CISPLATIN, EPIRUBICIN WITH / WITHOUT IFOSFAMIDE IN CHILDREN WITH BONE SARCOMA

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The toxicity of a new chemotherapeutic regimen consisting of cisplatin (CDDP, 100 mg/m^2/d), epirubicin (90 mg/m^2/d) with/without ifosfamide (2 gr/m^2/dx3) administered every 3 weeks,was evaluated in children (< 16 years of age) with newly diagnosed osteosarcoma (n=26) and mesenchymal chondrosarcoma (n=5). Of 147 courses administered, 46 consisted of CDDP and epirubicin; 101 included ifosfamide in addition to CDDP and epirubicin. Modified National Cancer Institute Common Toxicity Criteria was used as a guide to determining the grade of toxicities. In 106 (72%) courses various grades of anemia developed of which 57 (39%) required red cell transfusions. In 71 (48%) courses leukopenia occurred and colony stimulating factors (G-CSF or GM-CSF) was given for the treatment of 44 episodes. Hospitalization for empiric treatment of febril neutropenia was encountered in 18 (12%) courses. Three episodes of documented sepsis developed, 2 of which resulted in death. Trombocytopenia occurred in 44 (30%) courses and 28 of them required transfusions. Genitourinary toxicity was observed in 26% of courses. In spite of intensive antiemetic treatment severe nausea and vomiting occurred in 56% of courses. Ten episodes of hypocalcemic and/or hypomagnesemic tetany and 4 episodes of seizures associated with hyponatremia occurred. There was no cardiotoxicity and hepatic complications was minimal. Hearing could not be assessed regularly but 2 patients demonstrated severe ototoxicity. Overall the acute toxicity was serious in patients receiving three drug combination and intensive supportive treatment was required in order to treat the complications and to maintain the dose intensity.

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Osteosarcoma: Improved Survival and Reduced Late Toxicity

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The efficacy of a novel drug regimen designed to minimise late toxicity was evaluated. From 1988-95 17 patients (8 male, 9 female), median age 12.7 years (range 6.7 - 18) were treated. 3 patients had metastases at presentation. Primary sites included tibia (7), femur (3), fibula (3), humerus (3) and rib (1). 4 patients had amputation. Treatment comprised 5 cycles (30 weeks) of CEEV (carboplatin, epirubicin, etoposide, vincristine) alternating with IVA (ifosfamide, vincristine, actinomycin D). 87% (64/73) of CEEV courses and 89% (64/72) of IVA courses were given at full dose. Dose modifications of 20% - 50% were made for myelotoxicity, nephrotoxicity or SA > 2.0m^2. There was disease progression in 3 patients (6, 8 and 18 months) for an EFS of 78% with 2 deaths (15 and 22 months) for a survival of 84%. 15 patients are alive (14 disease free) at a median of 47 months (range 3 - 95) Serial echocardiography has documented no cardiotoxicity. 1 patient had a significant, but transient reduction in GFR, however, prolonged oral electrolyte supplementation was often required. In conclusion this pilot treatment regimen is highly effective, but without the long-term cardiac and renal toxicity observed with more commonly used adriamycin/cisplatinum regimens and warrants formal investigation in a randomised trial against existing more toxic treatment protocols.

P-114

EWING'S SARCOMA OF THE PELVIS

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Purpose: To optimize treatment strategy analysis of patient (pt) and treatment characteristics of Ewing'sarcoma in a difficult site, the pelvis, was performed.
Methods and Materials: From 1967-1995 30 new pt with Ewing's sarcoma of the pelvis were treated: ilium 19, ischium 5, pubis 6, sacrum none. Follow up: from 12 to 321 months (mo). Male-female ratio 13:17. Age range 3 to 18 years (yr), median 13 yr. Ten pt had metastases (mets) at diagnosis (9 lung, 1 bone). Local treatment consisted of radiotherapy (RT) in 26 cases, while after 1983 surgery was performed in 4 of 17 pt (2 metastatic). A dose of 40-45 Gy was given to a large field with a boost up to 55-60 Gy. From 1983 CT based RT planning was applied. Chemotherapy (CH), Dactionomycin, VAC or VACA started concomitantly or 6 weeks before RT in 13 pt; after 1983 CESS protocols were used with local treatment postponed to week 16, later week 12. Lung RT was given to all 9 pt presenting with lung mets, and in 3/8 pt with subsequent lung mets, while 4 had surgery (2 both). RT dose was 15-20 GY with a local boost.
Results: Overall survival (S) at 5 and 10 yr is both 30%. For the 20 M0 pt PFS and S at 5 yr is 20% and 35% respectively. Of 10 M1 pt, 1 is NED at 85 mo, 1 NED 22 mo, and one died NED at 16 mo. Of the 20 Mo pt, 12 developed mets (8 lung, 3 lung and bone, 1 bone), 7/8 before 1983 and 5/12 thereafter (p = 0,07). Two are alive >15 years.
Local results: One of 4 surgical pt had local failure and 9 out of 23 pt with local RT and FU > 1 yr, 2/10 before 1983 (wide APPA fields) and 7/13 thereafter (CT based planning). The relationship between tumor size, remission after induction CH, treatment technique and late effects will be presented.
Conclusion: 33% of pt presented with distant mets and 60% of M0 pt had subsequent mets. Survival after lung mets is possible. With CESS protocols metastatic rate decreased, but local control did not improve.

P-115

Analysis of a monocentric experience of surgery in Ewing's sarcoma. About hundred cases - 1979 - 1993.

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With the improvement of the outcome of patients with Ewing's sarcoma, local recurrence following radiotherapy have become a major concern advocating the wider use of surgery.
METHODS AND PATIENTS : From 1979 to 1993, we have seen 100 patients with Ewing's sarcoma whose were operated by our surgeon. Locations were ilium (6), spine (9), rib (17), femur (30), tibial (14), fibula (9), humerus (8) others (7). Metastases were present at the initial evaluation in 12. 25 patients were seen for local relapse after radiotherapy. Tumoral volume was > 100 ml in 69 cases.
The induction chemotherapy varied form 1979 to 1993 : 1979 to 1984 vincristine - adriamycin - cyclophosphamide - actinomycin (VACA) 3 months, 1982-85 CPX - THPADR (cyclophosphamide - tetrahydropyranyladriamycin) 3 months, 85-93 CPX - THP ADR 6 weeks. We added ifosfamide (IFX) and cysplatin (CDDP) from 1985 in the postoperative phase. Surgery was tumoral reduction in 16, en bloc resection in 84 (74 extratumoral, 10 contaminated).
RESULTS : Surgery was easier after short induction chemotherapy compared to long preoperative phase including radiotherapy. Eight local relapses were seen : 6 after contaminated or incomplete resection in spite of radiotherapy (out of 26). Of 74 en bloc extratumoral resection, we saw only two local relapses (3 %). **The surgical approach** of the Ewing's sarcoma **must be extratumoral**. Surgery is not able to salvage radiotherapy relapses and could be only palliative in such cases. Perioperative chemotherapy is feasible in the 24 hours following surgery. To avoid wound healing, ifosfamide 6 g/m² is the best tolerated.
CONCLUSION :
1) **Local control of the primary is better obtained by en bloc extratumoral resection than radiotherapy**. Contaminated or incomplete resection must be avoided. 2) The best way to permit extratumoral resection is the **Hayes's induction** (cyclophosphamide 7 d + THPADR 1 d) and the surgeon's experience. 3) Impact of surgery on disease free survival is significant early if resection is made after short induction < 2 months. Analyses of effect of local surgical control must separate clearly extratumoral or contaminated surgery, only and late surgery to avoid wrong conclusions on the role of surgery in Ewing's sarcoma.

P-116

Ewing's sarcoma: Late effects of radiotherapy on function and quality of life

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As long-term survival after Ewing's sarcoma increases (50%) the late effects of treatment become more important. The management of local disease is moving away from radiotherapy towards surgery. We assessed late effects of local treatment which included radiotherapy, alone or with surgery.
Between 1983 and 1993 sixty patients received radiotherapy (45-55 Gy): 22 are still alive (average follow-up 77.8 months). 9 received radiotherapy, 13 radiotherapy and surgery. We are comparing this group with controls, who received surgery only, using a modified Toronto Extremity Salvage Score questionnaire and a functional rating scale.

Results:

	Radiotherapy	RT & surgery
Upper limb	0	2
Lower limb	4	1

One of out seven require regular analgesia. One out of 8 is registered disabled. The choice of job was dictated by limb function in 4. Lower limb radiotherapy limited heavy household chores (4/5), sitting (3/5), stairs (4/5), and sports (5/5). Walking distance is affected (5/5) by stiffness and limited movement. Upper limb irradiation caused weakness-related limitations with carrying and lifting (2/2), sports (2/2) and heavy household chores (1/2). Problems with gait, skin texture, scarring and fractures occurred in 1 out of 7. Limb shortening (more than 5 cm) occurred in 2, and wasting (more than 5 cm) in 5. Shoulder flexion, abduction, internal and external rotation (1/2) and hip flexion, internal rotation (3/5), abduction (2/5) and external rotation (1/5) were affected. Careful attention to local therapy is a vital element of Ewing's sarcoma management requiring multi-disciplinary. The degree of deformity does not necessarily correlate with disability, and, surprisingly, all our patients experienced an improvement in function with time.

P-117

EWING'S SARCOMA/pPNET: 10 YEAR EXPERIENCE AT THE HOSPITAL FOR SICK CHILDREN (HSC), TORONTO

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Combination chemotherapy and radiation therapy is effective in the treatment of Ewing's sarcoma (ES)/pPNET but causes significant morbidity with a high incidence of second malignancies. Thus a protocol was designed using preoperative chemotherapy and surgical resection. From 1984 to 1994, 30 children with ES and 14 with pPNET were seen, 21 females and 23 males, age range 1-16 years. Overall, 33 of the 44 children (75%) are disease-free (NED) from 14-142 (median 71) months: 26 of 28 (93%) localised non-pelvic, 1 of 3 (33%) localised pelvic, and 5 of 12 (42%) metastatic. The protocol for patients with localised disease consisted of preoperative chemotherapy, surgical excision and reconstruction (if required), followed by postoperative chemotherapy, with radiation therapy limited to unresectable disease. From 1984-87, patients received Vincristine (V), Actinomycin (A), Cyclophosphamide (C) and Doxorubicin (Adria), In1986 Iphosphamide and VP16 (IP) were added. Since 1987 all patients received alternating cycles of VAdriaC and IP for a total of 12 cycles, from 2 to 4 cycles were given preop. Adria was given by continuous infusion to a total of 360mg/m2. Of 28 patients with localised non-pelvic disease only 5 required radiation therapy, 3 by intent (2 orbital and 1 vertebral tumor) and 2 for incomplete resection. All 5 are NED at 32-138 (med 110)mo. In 23 patients radiation therapy was avoided, 21(91%) of these are NED at 14-142 (med100) mo. Resection of a non-expendable bone was followed by immediate reconstruction, including of mandible and zygoma. Functional and cosmetic results were good in all cases. 1 early patient had a below-knee amputation because young age precluded radiation therapy. No difference in survival was found between ES and pPNET. The major toxicities were mucositis, fever and neutropenia. No cardiac or long-term toxicity was seen and no toxic deaths occurred. Excellent and functional disease-free survival is possible utilising neoadjuvant chemotherapy without radiation therapy or mutilating surgery, in localised non-pelvic Ewing's and related tumors.

P-118

EWING'S SARCOMA (ES): TWENTY-YEAR EXPERIENCE WITH 87 CHILDREN TREATED AT A SINGLE CENTER.

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A retrospective study was carried out in order to asses prognostic factors for long-term DFS in ES patients. We studied sex, age, location of the tumor, major diameter (CAT), LDH, seric albumin, WBCC, presence of systemic symptoms, performance status (ECOG), metastases, local therapy, protocol, pathology and clinical response to chemotherapy. Univariate Mantel-Cox, Cox-model (multivariate) and Kaplan Meier's were used as statistical methods. Between January 1970 and December 1993, we treated 87 patients (57 males, 30 females) under the age of 18 (median 12 years). The most frequent primary sites were the pelvic bones (20 cases), femur (13), chest wall (13), tibia (9), and vertebrae (8). Eight (9.2%) patients presented extraosseous lesions, and 15 (17.2%) were metastatic at diagnosis. Median serum LDH level was 254 U/l (88-1,400). Tumor size was greater than 10 cm in 45.2% of the series. Standard chemotherapy protocols were given in 75 cases (50.6% included a neoadjuvant phase). Local therapy consisted of surgery (27.6%), irradiation (37.9%), or both (29.9%). Median follow-up is now 63 months (12-212). Actuarial (Kaplan-Meier's) DFS at 5 and 10 years is 40.8 and 34.9%, respectively, with a median DFS of 25 months. A univariate (Mantel-Cox) analysis identified raised LDH levels in serum, hypoalbuminemia, metastatic disease, poor performance status, tumor size greater than 10 cm, axial location, elevated erithrocyte sedimentation rate, increased white blood cell count, systemic symptoms, and pathological fracture as poor prognostic features. Surgical treatment, neoadjuvant chemotherapy, and >90% chemotherapy-induced necrosis correlated with a better survival. The evolution of patients not achieving a complete response of relapsing thereafter was uniformly fatal. In a multivariate (Cox model) analysis, only serum albumin, LDH levels, and metastatic disease reached independent prognostic value. One-third of our ES patients become long-term survivors when treated with conventional combined therapy. In this retrospective series, high-risk patients were defined by the presence of hypoalbuminemia, high LDH levels and distan metastasis.

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OUTCOME OF PRIMARY EWING'S SARCOMA OF BONE OVER A DECADE (1983-1993)

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152 patients with primary Ewing's sarcoma of bone treated on the same protocol over a decade in a single centre were studied. Mean age was 17.5 years. 19 patients (12.5%) presented with metastases. Minimum follow-up was 32 months. The patients had chemotherapy according to UKCCSG (MRC) ET1 and ET2 protocol. Local treatment was surgery in 111 patients, surgery and radiotherapy in 9 patients and radiotherapy alone in 32 patients.

Disease-free and overall survival in patients without metastases at diagnosis were 59% and 66% respectively at 5 years. All the patients with metastatic disease at diagnosis died before 3 years. Outcome of patients with localised disease at diagnosis was dependent on chemotherapy-induced necrosis, tumour volume, ESR at diagnosis and surgical treatment. The 5-year survival of patients with localised extremity tumours was 71% compared to 42% for central tumours but the difference was statistically insignificant when tumour volume and local treatment were taken into account. Local recurrence rates following radiotherapy was 28% compared to 8% after surgery, and 5% after wide excision, 10% after marginal excision and 25% after intralesional excision and radiotherapy.

Tumour stage, necrosis and adequate surgical excision are the most important independent prognostic factors in primary Ewing's sarcoma of bone.

P-120

CHANGE IN TUMOUR VOLUME AS A MEASURE OF CHEMOTHERAPY INDUCED NECROSIS IN EWING'S SARCOMA OF THE BONE

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The CT/MRI scans and histology of 50 patients with primary Ewing's sarcoma of bone treated between 1983 and 1993 were reviewed to determine the correlation between change in tumour volume and tumour necrosis following chemotherapy; and determine the influence of tumour necrosis and change in tumour volume on prognosis. The mean age was 17 years. The extremities were involved in 40 patients and central bones in 10 patients. The volume at diagnosis varied from 31 to 1790ml.

A negative correlation was observed between the change in tumour volume and necrosis following chemotherapy (R=0.66, P<0.0001). Tumour progression, despite chemotherapy, was only seen in those with less than 60% necrosis. The relapse-free survival and overall survival were 75% and 78% respectively for those with more than 90% necrosis at 5 years (P=<0.05). The outcome of patients with more than 40% volume reduction was better than those with less than 40% reduction but this did not reach statistical significance.

We conclude that change in tumour volume is a good predictor of chemotherapy induced necrosis and necrosis is a strong prognostic factor in Ewing's sarcoma.

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DOSE AND TIME INTENSIVE CHEMOTHERAPY FOR EWING'S SARCOMA

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Six consecutive newly diagnosed children with Ewing's Sarcoma (ES), aged 6-15 years, were treated with a dose and time intensive chemotherapeutic regimen. Primary sites were: pelvic-2, femur-2, humerus-1, paraspinal-1. Three patients had metastatic disease (2 lung, 1 bone marrow). Five of 6 had tumors $\geq$ 8 cm in greatest diameter. Five of 6 pts had biopsy only at diagnosis and one had complete resection of the primary. Treatment consisted of high dose cyclophosphamide (CPM) 3 g/m^2 over 2 days and etoposide (VP-16) 480 mg/m^2 over 3 days on day 0 and day 14, regardless of counts, followed by G-CSF. The same drugs plus doxorubicin (DOX) 75 mg/m^2 were repeated on day 35 and 56. Vincristine (VCR) 1.5 mg/m^2 was given weekly x 6 beginning on day 35. Local control was instituted at day 77. Local control consisted of: 3 limb salvage alone, 1 limb salvage plus XRT, 1 XRT alone and 1 no further local control. Maintenance therapy consisted of CPM & VP-16 alternating with DOX & VCR every 4 weeks with recovery of counts required. The planned duration of therapy was 1 year. With a median follow-up of 2 years, five of 6 pts are alive, off therapy, with no evidence of disease. One patient died of pneumocystis carinii pneumonia and was free of disease on autopsy. The therapy is toxic, but tolerable and appears highly effective. Dose limiting toxicity was thrombocytopenia. Chemotherapy was given as an out-patient. Average number of hospital days was 40. Further studies in larger numbers of pts are indicated.

P-122

PROGNOSTIC RELEVANCE OF TUMOUR VOLUME IN EWING'S SARCOMA: THE INDIAN EXPERIENCE.

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A study was undertaken in 25 patients of biopsy proven localized Ewing's Sarcoma of the long bones during 1992 to 1995,to study the prognostic significance of primary tumor volume. There were 19 males (76%) and 6 females (24%) of which 88% were under 20 years of age. Non-metastatic status was confirmed by routine blood and biochemistry tests, chest X-rays, bone marrow examination and isotopic bone scans. Tumor volume was calculated from orthogonal plain radiographs. All patients received upfront chemotherapy with Vincristine, Cyclophosphamide, Doxorubicin with or without Etoposide, Cisplatinum or Iphosphamide in various schedules and local radiotherapy to 50 to 60 Gy with conventional fractionation and a shrinking field technique. Tumor volume was calculated prior to start of therapy and at completion of irradiation and was correlated with local control and survival. Nine patients had initial tumor volume of $<$100 ml (36%) and 16 of $\geqslant$100 ml (64%). After therapy, 7 cases showed a volume reduction of $\geqslant$50% (28%) while 16 patients of $<$50% (64%) and 2 cases recorded progression. Of the 9 cases with initial volume of $<$100 ml, 8 (89%) were locally controlled while of the 16 cases with $\geqslant$100 ml, local control was seen in only 10 patients (63%) (p=N.S). One patient (11%) in the former group developed local recurrence which occurred in 4 patients (25%) in the latter group. The overall median survival for the two groups of patients was 27 and 18 months respectively (p=N.S). Good correlation between tumor volume and clinical outcome of therapy was thus seen in these cases. The study is continued to analyse other prognostic variables in a larger group of our patients.

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TREATMENT RESULTS AND PROGNOSTIC FACTORS IN EWING SARCOMA

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Between January 1971 and December 1993, 112 consecutive children with newly diagnosed Ewing sarcoma (ES) were treated and followed up in a single center. There were 58 males and 54 girls, aged between 1 and 17 (median 10)years. The primary location of tumor was in extremity in 53.6%, trunk 27.7% and 12.5% pelvic sites. Thirty-five (31.2%) patients had metastatic disease at diagnosis. The most common site for metastasis was lung and bone, each 42.8%. Curative surgery has been done only in 16.6% of patients. The five year overall survival rate was superior for patients treated with doxorubicin or epirubicin containing VAC regimen (39.5%) compared to other VAC based regimens (25, 20.3 and 13%). Localized disease, low tumor volume and distal primary were found as good prognostic factors. The eight-year overall survival rates were 32% and 11.8% for localized and metastatic disease, 60.9% and 19.6% for patients with low and high volume disease respectively. The eight year event-free survival rates were 28.2, 21.8, 10.2% for patients with distal extremity, proximal extremity, trunk tumors respectively and no patient with pelvic tumors survived more than four years. The results showed us that ES in children should be treated with more intensive regimens and we have started to use such regimens for the last two years.

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EWING'S SARCOMA: THE INFLUENCE OF SYMPTOM DURATION ON SURVIVAL PROBABILITY.

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A retrospective analysis of 30 children with Ewing's sarcoma treated during the period between 1980 through 1994 was performed. There were 16 males and 14 females with a mean age of 10.5 yrs. According to the TNM classification, 19 pts had St I-II, and 11 pts had St III-IV disease (primary tumor 100cc in 11 pts; lung mets in 3 pts). The mean duration of symptoms prior to diagnosis was < 1 month in St I-II pts, and 4 months (range: 2-25)in St III-IV pts. The primary site was a limb in 12 pts, the ribs in 9 pts, the pelvis in 5 pts, the skull in 2 pts and a vertebra in 2 pts. Local treatment consisted of radiotherapy (RT) in 12 pts, surgery (S) in 9 pts and RT+ S in 9 pts. Twenty one ptstreated prior to 1986, received systemic chemotherapy using the VACA combination; 9 pts, referred after 1986, were treated according to the guidelines of the IESS protocol. The mean follow up time is 10.1 yrs. The following actuarial 5 yr survival figures were obtained:

St I-II (duration of symptoms < 1 month): 93%
St III-IV (duration of symptoms > 4 mos): 36% (p < .010)
Radiotherapy: 75%
Surgery 60%
Radiotherapy +Surgery: 75% (P=N.S)
Primary site:
Limb: 81%
Ribs: 71%
Pelvis 60% (P=NS)
Age:
< 10 yrs: 73%
> 10 yrs: 64% (P=NS)

It is concluded that stage and duration of symptoms prior to diagnosis are significant prognosticators of survival; age, primary site and type of local and systemic therapy did not significantly influence the 5 yr survival probability in our series.

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HIGH-DOSE CYCLOPHOSPHAMIDE FOR EWING'S SARCOMA (ES)

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Ewing's sarcoma occurs more frequently in children and adolescents and have a highly malignant behaviour. Most of the studies published using a standard dose regimen with vincristine (VCR), dactinomicin (ACT), doxorubicin (ADR) and cyclophosphamide (CTX) have shown long-term relapse free survival lower than 50%. This study analysed toxicity and response to an ambulatorial chemotherapy regimen using VCR (2mg/m² x1), ADR (30mg/m² x2) and high-dose CTX (1200mg/m² x2) plus MESNA (1200mg/m² x1), repeated at 21-day intervals during one year (17 courses). After reaching the cumulative dose of 350mg/m², ADR was replaced by alternating courses with ACT (1,5mg/m² x1) or Etoposide (200mg/m² x2). Following 3 courses of chemotherapy, CTX was given only on day 1 (50% dose reduction) for the remaining 8 courses. Toxicity (WHO) was assessed between the 10th and the 14th day of the cycle and just prior the next one. Response (WHO) was verified between the 12th and the 15th week of treatment (after the 4th cycle). Local treatment (surgery, radiotherapy or both) was performed when feasible (reduction of the primary tumour and desappearing of the metastasis), usually after the 4th cycle. From February/94 to February/96 nine patients entered in the study. The main characteristics are median age of 13 years (range 4 -19), sex ratio 4M/5F, stage at diagnosis local in 6 and metastatic in 3. From a total of 95 cycles administered, the major toxic effects were grade III-IV leucopenia in 45 courses, associated with grade III-IV infection in 23 episodes. So far, 7 patients were available for response and 5 achieved CR. Four patients completed the treatment. One patient relapsed with bone metastasis within 7 months of follow-up. The median follow-up period is 6 months (range 2 - 7). Although the short period of follow-up, this chemotherapy program appears to be an efficient option for ES patients, with tolerable toxicity.

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EWING'S SARCOMA IS A RARE DISEASE IN THE FAR EAST

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This study of the incidence of Ewing's Sarcoma (ES) in the Far East is prompted by the clinical impression of the rarity of this disease among Malaysian children and supported by results of the recently concluded Malaysian National Childhood Cancer Survey, 1993-95. Only 13 cases of ES (27% of all bone cancers) were diagnosed in children below the age of 15 years during the 3-year period, giving an incidence 0.6 per million. The osteosarcoma (OS)/ES ratio is 2.7. Similar ES incidence figures were reported in the WHO IARC report on the International Incidence of Childhood Cancer from Singapore (0.5), Hong Kong (0.5), Taiwan (0.5), Shanghai (0.7) and Osaka (0.6). Their OS/ES ratios ranged from 2.7 in Thailand to 7 in Singapore (average about 5.0 in the Far East). In contrast, the incidence of ES among western caucasian populations is much higher - 3 per million in Australia, 2.7 among the whites in the USA and 1.8 in Manchester. The OS/ES ratio is only 0.3 in Australia, 1.1 in USA Whites and 1.7 in Manchester.

There appears to be no explanation to the low incidence of ES in the Far East other than relative genetic resistance to the disease, similar to that seen in Africa and also among the Blacks in the USA.

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High-grade osteogenic sarcomas in the young occurring in the pelvic arch: a retrospective study of the French Society for Pediatric Oncology.

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From January 1980, to December 1990, 23 high-grade osteosarcomas with primaries in the pelvis or in the pelvic arch were observed in the SFOP Centers. The median age was 16 years (range 13-41) and the M/F sex ratio was 1/1. The localisation of the primaries was :11 ilium,1 ischium, 4 anterior pelvic arch, 4 acetabuli, and 3 sacrum. 4 patients had initial metastasis (2: lung, 1: lung+distant bone, 1: other). Most of the tumors were large sized tumors (> 100 mms). More than 50% of the tumors had an important histological chondroid component. 21 patients received initial chemotherapy (5: SFOP OS 87, 10: HELP Adriamycin, 6: others). 12 patients were operated; 1 of 12 received additionnal radiotherapy because of marginal surgery. 11 patients were only irradiated (4 by Cobalt therapy only and 7 with Cobalt+Neutrontherapy). With a median follow-up of 30 months, the overall survival is 75% at 48 months for all patients. There is no difference in overall survival between the patients who were operated and those who were irradiated (71% vs 87%). The progression-free survival is however better for patients who underwent radical surgery than for patients who had only radiotherapy for the treatment of the primary tumor (PFS = 50% vs 19% at 32 m, p=NS)
Despite aggressive chemotherapy in most cases, the prognosis of osteosarcomas of the pelvic arch in the young is poor, and largely worse than the prognosis of osteosarcomas of the limbs in the same age category. The presence of a frequent chondroid component in these tumors as well as the non-feasability of surgical "en-bloc resection" of these large tumors are potentially poor prognostic factors. However, despite a low DFS or EFS rate, aggressive chemotherapy and surgical procedures may lead to an improved outcome.

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OSTEOSARCOMA:
THERAPEUTIC STRATEGIES, RESULTS AND PROGNOSTIC FACTORS.
EXPERIENCE OF THE NATIONAL CANCER INSTITUTE, CAIRO, EGYPT.

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During the period of 1990-94, 60 pediatric patients were treated with two separate protocols: osteosarcoma Study Group (OSG) I and II. Seventeen patients developed metastases. Mature data from these protocols are available. Overall 5 year disease-free survival (DFS) was 64± 7.5%. In OSG I, (1990-1992), 36 patients were treated with an adjuvant chemotherapeutic regimen comprising epirubicin and cisplatin. Primary definitive treatment comprised amputation. Five year disease-free survival (DFS) was 60± 9.5%. In OSG II, (1992-1994), 24 patients were treated initially with a preoperative chemotherapeutic regimen comprising epirubicin and cisplatin followed by surgery. Surgery comprised amputation or limb salvage. The five year DFS was 71± 11.8%. There as no difference in DFS between patients undergoing amputation or limb salvage. Factors considered significant in relation to the development of metastases comprised tumor burden ($p<0.01$) and percentage of tumor necrosis induced by preoperative chemotherapy ($p<0.01$). These findings are consonant with results and prognostic factors published in the literature. Based upon these experiences, a more intense protocol, OSG III was opened in 1995 for patient entry. It comprises preoperative Ifosfamide, Adriamycin and high-dose cisplatin (150 mg/m^2). Postoperative chemotherapy comprises high dose Methotrexate, Ifosfamide, and Adriamycin. Thirty-five patients have been entered. Provided sufficient mature information can be assembled, results from this protocol will also be presented.

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OSTEOSARCOMA (OS) OF THE JAW IN CHILDHOOD. A CLINIC, RADIOLOGIC, AND PATHOLOGIC STUDY.

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OS of the jaws usually presents one to two decades later that those of extremities, their clinical behavior is caracterized by slow local growing, intramedullary localization, low grade histology, multiple local recurrences and late metastatic dissemination with long survival. Between Dec 1956 and Dec 1995, 14 patients (pts) with OS of the maxillary bones are described. Age ranged from 6 to 17 years (m 12y), with male predominance (4:3) and upper maxilla (4:3). In 1 case the maxilla OS had and antecedent of a primary OS in femur 7y before, which was treated with surgery and chemotherapy. No other predisposing factors, as prior radiotherapy, retinoblastoma, fibrous dysplasia, familiar history or anothers were found. The presenting symptoms were mass and pain. Radiologic imaging was nonspecific, there was predominance of lytic lesions and only 2 pts presents with sunburst pattern. In all the cases there was a bulky tumor with extraosseous mass located intramedullary and of high grade histology (grade 3:4); predominantly chondroblastic type in upper maxilla an fibroblastic in mandibule. The treatments administrated were not uniform, surgery alone with or without chemotherapy and/or radioterapy were perfomed in the majority of the pts. The surgical radical margins were technically difficult to obtain in the upper maxilla, whereas the local relapses were more frequent. Metastastic dissemination was to the lung in 2 pts at 7 and 30 mo. Six, pts dead: 3 due to surgical complications and 3 of neoplastic disease; 3 pts were lost for follow up and the 5 remainder are alive without disease with a follow up between 1-30 mo.

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COMPARATIVE RESULTS OF OSTEOSARCOMA (OS) TREATMENT WITH AND WITHOUT HIGH DOSE METHOTREXATE (M) IN A SINGLE INSTITUTION

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From April 83 to August 95, 50 patients (Pt) with localized (IIB) extremity OS (conventional 43, telangiectatic 7) were treated in the Italian Hospital. Median age: 16 years (6-23), 32 males. Localization : femur 32, tibia 12, fibula 4, humerus 2 . All had chemotherapy (Cx) before and after surgery in 3 consecutive protocols : Cisplatinum (P)+ Adriamycin (A) (Petrilli S, ASCO 5,1986) or P+ Ifostamide (I)+A (Richard L, Med Ped Oncol 17, 1989) or M + I + A (Miser J ASCO 9,1990). Pt were divided in 2 groups (G) according year of treatment and protocol. Ga (83/90) 19 Pt PA or PIA ; Gb (90/95) 31 Pt MIA. Both G were comparable (age gender, localization, histology). Median time of follow up was: Ga 60 months (m); Gb 29 m. Median time Cx before surgery was shorter (p=.001) for Ga 78 days (53-235) vs.Gb 126 days (19-198). Local treatment: amputation (Am) (Ga 9 / Gb 3) or limb salvage (Ls) (Ga 10/ Gb 28). Difference of local treatment between groups were significant (p=.0019).In Ga 13/19 Pt relapsed and in Gb 9/31. Site of relapse : local (Ga 0/Gb2); local + lung (Ga 1/Gb 2) ; local + other (Ga 1/Gb 0); lung (Ga 9/Gb 3); lung + bone (Ga 2/Gb 2). Local relapse was associated with Ls (p=.0000). Systemic relapse was more frequent (p=.0009) in Ga vs. Gb. Pt with local and/or lung relapses had better (p=.029) overall survival (S) (32% at 36 m) vs. those with lung +bone or other (11% at 58 m) . Pt with Am had more systemic relapses (p= .03) vs. Pt with Ls. Disease free survival (DFS) (20 % at 23 m) and S (20% at 48 m) in Pt with Am were very poor in both G. In Pt with Ls DFS was better (p=.0024) in Gb (68% at 33 m) vs Ga (24% at 31 m); S was similar (p=.29) in both (Ga 58% at 60 m vs. Gb 63% at 45 m).
Conclusion: Pt with Ls and MIA had better DFS than those with Ls and PA or PIA. Nonetheless Pt with Ls relapsed from PA or PIA had a similar S than those with MIA because they can be rescued with other aggressive Cx. On the other hand Pt who had systemic relapse after MIA or Pt who needed Am after any Cx had a very poor prognosis.

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COMPARISON OF PRIMARY OSTEOSARCOMA (OS) OF FLAT BONES WITH SECONDARY OS OF ANY SITE.

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Secondary OS of any site are generally high grade lesions, for which the prognosis is poor. OS of flat bones often involve regions that cannot be completely resected without significant morbidity. In addition to 290 primary OS of long bones, we have treated 25 primary tumors of flat bones and 18 secondary OS. Primary OS of flat bones involved 12 males; median age of patients (pts) was 14.9 yrs (range 3.2-24.9 yrs); predominant sites were the pelvis and head/neck; 17/25 were unresectable at diagnosis; 9/25 survive 2.0-13 + yrs (Median = 3.9 yrs); receiving treatment for active disease; including 2 pts presently receiving treatment for active disease; median survival for those who died was 1.2 yrs (range 0.6-3.3); causes of death were tumor in 15, cardiomyopathy in one. Among the 18 pts with secondary OS, the primary tumors were retinoblastoma 7, Hodgkin 4, rhabdomyosarcoma 3, and one each with acute lymphoblastic leukemia, astrocytoma, synovial sarcoma and OS; median age at primary diagnosis was 2.8 yrs (range 0.0-5.9 yrs), at secondary OS 14.2 years (range 9.7-33.9 yrs), with a median interval of 10.7 yrs between primary tumor and secondary OS (range 6.5 - 23.5 yrs); 15 pts had prior radiation therapy including one pt who had TBI prior to syngeneic BMT (median dose = 3800 cGy, range 1200 - 6480 cGy); 14 of the lesions developed within the portals of previously delivered radiation therapy; sites of secondary OS were the head/neck ($n=7$) and pelvis ($n=3$); 9/18 were resectable at diagnosis; 2 OS were multifocal; 2/18 pts survive disease-free at 5.5 and 10.3 yrs and one is living with disease at 6 months; for those pts who died, median survival time was 0.9 yr (range 0.1-5.7 yrs); cause of death were the secondary OS (14), intratumoral hemorrhage (1), and cardiomyopathy/coccidiomycosis (1); one pt with primary Hodgkin disease had a third malignant neoplasm discovered at autopsy. The one-year overall survival for primary flat bone OS was 71% (± 12%) compared to 47% (± 10%) for the secondary group (p=0.22). There exists significant need for improvement in outcome for both groups. (Supported by *USPHS Grants CA-23099, CA-21765 and by American Lebanese Syrian* Associated Charities [ALSAC]).

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RETROSPECTIVE ANALYSIS OF 154 OSTEOSARCOMAS TREATED AT THE CENTRE LEON BERARD SINCE 1979.

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154 patients (pts) (83M, 71F) with osteosarcoma were treated in Centre Bérard since 1979. Median age is 17 (range 4-91). Tumors sites were: femur (47%), tibia (25%), humerus (10%), flat bones (13%).With a median follow-up of 9 years, the 5, 10 and 15 years overall survival (OS) are 59% ,51% and 46% respectively. The last relapse and the last death due to disease occured at 63 and 138 months after diagnosis respectively. In univariate analysis, prognostic factors for OS at diagnosis were, HUVOS score (p=0.0001), unfavorable tumor site (UTS), i.e.flat bones, humerus or upper extremity of femur (p=0.0006), increased alkaline phosphatase level (AP) 5-fold above normal (p=0.01), age>40 (p=0.02). In multivariate analysis using Cox model, Huvos score (p=0.0001), UTS (p=0.002) , and AP levels (p=0.04) were identified as independent prognostic factors. The type of chemotherapy (CT) regimen (*no CT* vs *T7-T10-T12* vs *ifosfamide-CDDP regimens*) were then included in the multivariate model after adjustment on the 3 above defined prognostic factors: ifosfamide-CDDP containing protocols were independently correlated to OS (p=0.007).
The median OS from the date of relapse of the 61 relapsing pts was 16 months and 5 years survival 19%. Using Cox model, a localized relapse, a diagnosis-relapse interval>12 months, and an initial tumor site different from UTS had an independent prognostic value for OS after relapse (p<0.05). Two year OS from relapse of pts with none, 1,>1 of these 3 risk factors are 100%, 50% and 13% respectively.

P-133

PROGNOSTIC FACTORS IN NONMETASTATIC LIMB OSTESARCOMA: A 20-YEAR EXPERIENCE OF ONE CENTER

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The survival of patients with has improved significantly, primarily due to combination and neoadjuvant chemotherapy. To identify prognostic factors in osteosarcoma and assign the patients to different treatment regimens, we analyzed the results in 35 consecutive patients with non-metastatic osteosarcoma of the extremities that were treated in our center between 1973 and 1993. The following variables were evaluated: age, sex, ethnic group, tumor histology and primary site, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels at diagnosis, treatment regimen, methotrexate (MTX) levels after 24 and 48 hours, and the histologic response to therapy. Four variables had correlated with prognosis: histologic response to treatment: DFS at 64 months (mths) was 89% in patients with histologic response grade III-IV, 67% in patients with grade II, and 0% in patients with grade I, p<0.0001; treatment regimen DFS was 83% at 42 mos, 62% at 82 mos, and 30% at 177 mos in patients treated by Protocols 90's, Protocol 80's, and Protocol 70's respectively, p<0.05; corrected ALP (cALP) levels at diagnosis DFS was 78% at 88 mos in patients with cALP levels < 200, and 32% at 56 mos in patients with cALP levels < 200, p+0.01; (4) DFS was 75% at 56 mos when MTX levels were higher than 150x10-7 mole/L at 24 hrs, and 40% at 76 mos when MTX levels were <150x10-7 mole/L, p=0.05).
It is concluded that low cALP levels, good histologic response, high MTX levels, and intensive chemotherapy, correlate with good prognosis in osteosarcoma.

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IFOSFAMIDE/ETOPOSIDE ALTERNATING WITH HIGH DOSE METHOTREXATE - EVALUATION OF A CHEMOTHERAPY REGIMEN FOR THE TREATMENT OF RELAPSED AND METASTATIC OSTEOSARCOMA

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We report the results of a new combination schedule in patients with relapsed or metastatic osteosarcoma, treated at 2 UK centres. Therapy was commenced with *ifosfamide 2.5 g/m² daily x 3 days, etoposide 150 mg/m² daily x 3 days and high dose* methotrexate 8-12 g/m² (with folinic acid rescue) on day 10-14 in a planned 21 day cycle. Surgery was performed when appropriate.

Patients: 17 patients were evaluated (7 males, 10 females; age 6-24 years, median 14 years) 13 had relapsed disease, 2 had osteosarcoma as a second malignancy and 1 had primary metastatic disease. All bar 1 had received first line therapy of cisplatin and adriamycin according to MRC/EORTC osteosarcoma guidelines. 2 patients had tumour surgery at presentation and received the regimen as adjuvant therapy.

Responses: 15 patients were evaluable for response. 5 of 9 with pulmonary metastases achieved PR after 2-10 courses; and 1 had a radiological CR after 10 courses. 2 had SD and 1 PD. 5 patients with PR/SD had thoracotomies and converted to CR. 4 patients had bone metastases either alone (2) or in combination (lung-1). (lung and CNS -1). 2 of these achieved PR after 3-10 courses; 1 had SD after 2 courses but refused further treatment; 1 progressed after 3 courses. 1with a 2nd malignancy had PR at the primary site after 3 courses with >90% histological tumour necrosis and achieved CR with tumour surgery. 1 with a limb primary and multiple pulmonary metastases at diagnosis achieved PR at both sites after 4 courses and remains on treatment. 7 patients are alive with no evidence of disease, 8-75 months post entry into study. 4 are alive with radiological evidence of disease (3-19 months). 6 have died from disease. 7-54 months later. None died a toxic related death.

Toxicity: There were 107 evaluable courses. The mean in-patient stay was 11.1 days per cycle (min 5, max 27). 15 courses were complicated by infection of grade 1 severity; 46 courses with grade 2; 3 courses with grade 3. Grade 1 renal complications were seen in 23 courses; grade 2 in 2 courses and grade 3 in 1 course. Haematological toxicity of grade 3 or 4 was seen in all courses. Most courses required phosphate and magnesium supplementation.

Conclusion: This regimen exhibits an encouraging response rate (66%). in a group of children with poor prognosis disease with a tolerable toxicity profile.

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FAILURE OF HD-MTX IN IMPROVING SURVIVAL OF OSTEOGENIC SARCOMA PATIENTS WITH POOR PROGNOSIS.

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For 2/3 of the world population that lives in the so called developping countries, late diagnosis resulting in higher tumor volumes comprises at least 50% of the OS patients. From Oct/91 to Dec/95, we registered 123 pts in a Colaborative Brazilian OS Study III, with 80 nonmetastatic extremities evaluable patients. They were treated with a intensive combination of Ifosfamide/Carboplatin, Ifosfamide/Epirrubicin and Epirrubicin/ Carboplatin, followed by surgery. During maintenance, patients who underwent amputation or the tumor diameter after pre operative chemotherapy was $\geq$ 12 cm (48%), were considered based in our previous experience as poor prognostic and were intensified with 6 doses of HD-MTX (12 g/m2) together with the same pre operative chemotherapy repeated in 2 cycles (Reg. b). 52% of the pts (tumor diameter $\leq$ 12 cm) received the same regimen without HD-MTX (Reg. A). The CCR index for the study was 40% higher for the Reg. A vs Reg. B (74% vs 54%) with a median follow-up of 23,2 months. (3-50 m). The patients with tumor diameter $\geq$ 12 cm, presented less conservative surgeries (42% vs 84%) and the degree of more intensive necrosis (III and IV) were found in 24% vs 63% for the other pts. This experience suggests that even an intensive chemotherapy regimen with the addition of HD-MTX cannot rescue these significant patient population with large tumors and there is a need of new treatment strategies to overcome resistant in order to increase the survive index besides the educational programs.

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ESTIMATION OF TREATMENT RESULTS OF METASTASES IN OSTEOGENIC SARCOMA IN CHILDREN AND ADOLESCENTS.

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Between 1985 and 1994, 158 osteogenic sarcoma children were treated. The age ranged from 3yrs to 25yrs.
Distant metastases were detected in 34 patients: at diagnosis-7; during the course of treatment-13 (within 6mos from diagnosis-6; after 6mos from diagnosis-7);after the end of the treatment-14(between 6 to 36 mos).
The lung metastases were found in 32 cases (in 10 bilateral), In this group additional sites of distant spread were detected: femur-3, rib-1, neck spine-1, lumbar spine-1). Isolated bone metastases were shown in 2 cases (pelvis, femur, madible), and CNS spread in 1 patient.
The treatment was initiated with chemotherapy according to different protocols. There were 3 groups of patients:
Group I-chemotherapy + surgery of the primary + adjuvant chemotherapy; II-chemotherapy (only in 1 patient the surgical approach for pulmonary lesions); III-chemotherapy + thoracotomy + chemotherapy
The following types of thoracic surgery was performed in 18 patients: unilateral thoracotomy in 8 patients(3 times in 1 patient); sternotomy in 5 (2 times in 2 patients). Bone metastatic lesions were resected in 2 patients, laminectomy was done in 2 cases and brain metastasis removal in 1 patient.
RESULTS
In the group of 16 children, where conservative treatment was employed, only 2 patients survived, while in 15 operated ones, 10 patients were survivors. Surgery is planned in 4 patients.
CONCLUSIONS
Removal of metastases at the time of partial remission or stabilisation after neoadjuvant chemotherapy seems to be the treatment of choice in osteogenic sarcoma. Repeated lung metastatectomies (2-3 times) can prolong survival.

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CURE OF OSTEOSARCOMA, AT WHAT PRICE?

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Osteosarcoma is the most frequent primary malignant bone tumor. From the early 1970s effective chemotherapy regimens have been used in the treatment, improving disease-free survival up to 75%. The protocol used in the Netherlands is the EORTC protocol containing a two drug regimen of doxorubicine (25 mg/m2 x3) and cisplatinum (100 mg/m2) given at a 3 week interval 3 times preoperative and 3 times postoperative.
We reviewed the short-term side effects of this therapy of 22 patients treated during the period of 1984-1995. We analised the cardiotoxic, nephrotoxic, ototoxic side effects and the infection rate. In 4 patients (18%) echocardiograms showed an evident decrease in the left ventricular contractibility and a significant deterioration in the shortening fraction. In those patients doxorubicin therapy was stopped. In 11 children (50%) the creatinine clearence was between - 1 and -2 SD, but this was not a reason to stop therapy. The audiograms of 6 patients (41%) showed an evident degree of high-frequency hearing loss (between 45 and 65 dB), which led to a dose reduction in 1 case, stopping of cisplatinum in 4 cases, and replacement of cisplatinum by carboplatin in 4 cases. In 22 patients 28 episodes of fever were observed during an aplastic period. A positive blood culture was found in 7 episodes (25%). More often side effects of therapy led to hospitalisation of the children than the administration of chemotherapy. Of the 22 children only one had an event free course of treatment. 3 patients (14%) had metastatic disease at diagnosis, they all died. 6 patients (27%) developed metastatic disease during or after therapy, 2 of them are still alive. 13 (60%) patients are disease free. None of the patients died because of side effects of the therapy. The cost of increasing survival rates of osteosarcoma are high due to frequent and serious side effects which adversely influence the quality of life.

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OSTEOSARCOMA: COMPARISON BETWEEN TWO METHODS FOR POST-CHEMOTHERAPY NECROSIS QUANTIFICATION

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Forty two patients with high grade osteosarcoma were analyzed for response to chemotherapy (Huvos grading) by two methods: GRID (usually practice) and CAD (new method using computer image analysis). The GRID method is easy to be done but overdiagnosed viable tumor areas. The CAD (Computer Aided Design) method is very practical, easy to be done and more precise in quantifying tumors necrosis. The concordance between these two methods was verified by Kappa analysis and showed an agreement of 60%, excluding the random effect. Some prognostic factors and survival were also analysed. On univariate analysis by Kaplan-Meier method, the most significant predictors on survival were: age, bone involved, site in the bone and response to chemotherapy by CAD method. After the multivariate analysis using the Cox proportional hazard model, only the physis involvement and necrosis degree were retained as independent predictors on survival. Good responders (Huvos III and IV) had overall survival of 60.6% in 10 years. Bad responders (Huvos I and II) had overall survival of 30.7% in 5 years and 15.3% in 10 years.

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OSTEOSARCOMA IN CHILDREN AND ADOLESCENTS : A SINGLE INSTITUTE EXPERIENCE OVER 10 YEARS.

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We reviewed our results of osteosarcoma in Queen Mary Hospital, Hong Kong from 1985-1995. The aim is to see if the favourable treatment results in experienced centres can be replicated in a local oncology centre with limited exposure to this uncommon disease. There were total 24 patients, 14 males and 10 females, aged 20 or under at diagnosis. 19 had localised disease and 5 presented with metastases. Except for one Vietnamese, all others were Chinese. All had limb primaries. All patients received multi-agent chemotherapy. For patients with localised disease, 17 patients had neoadjuvant and adjuvant chemotherapy. 2 patients had adjuvant chemotherapy only. The chemotherapy scheme varied but was based mainly on Rosen T10 protocol. One patient had limb salvage surgery while all others had amputation. The actuarial 5 year disease free and overall survival were 64.5% and 66.3% respectively, with median follow-up of 35 months. Females and high post-infusion methotrexate level, more than 900μmol/L, were found to be good prognostic indicators. 4 of the 5 patients presented with metastases died of disease. One patient with lung secondaries at diagnosis is a long term survivor for 125 months after amputation, chemotherapy and multiple thoracotomies. We concluded that osteosarcoma can be adequately managed in a local oncology centre with surgery and intensive multi-agent chemotherapy. The favourable survival results in experienced centres are replicable in the local settings. Further development in expertise would be required to improve the quality of life and functional ability of survivors of this childhood malignancy.

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ADOPTIVE IMMUNOTHERAPY (PBMC+rIL2) IN ASSOCIATION WITH CHEMOTHERAPY (CT) AS PRE- AND POST-OPERATIVE TREATMENT FOR CHILDHOOD OSTEOGENIC SARCOMA (OS)

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Several studies on OS showed that modifications of therapeutic regimens, as to timing and selection of drugs, were of little benefit in improving the prognosis. Clinical and experimental evidence supports the concept of associating immunotherapy (IT) with CT, even though a clear-cut synergism has not been demonstrated as yet. In 1991 we started a therapeutic program in which IT with rIL2 and peripheral blood mononuclear activated cells (PBMC) was given in association with the same pre- and post-operative CT regimen adopted between 1986 and 1990 for childhood OS. Treatment consisted of rIL2 ($9x10^6$IU/m^2/daily x 4) followed by leucapheresis, 2 monthly cycles of VCR (1.4 mg/m^2) + MTX (8 g/m^2 weekly x 2) and CDDP (40 mg/m^2/daily x 3) + IFO (1.5 g/m^2/daily x 3), and one cycle of rIL2+PBMC. After surgery, CT was piloted by the histopathologic response (necrosis ≥90%: as above for 1 cycle; <90%: ADM 30 mg/m^2/d x 3 days x 5 monthly cycles). The treatment program ended with rIL2+PBMC. Pts with synchronous lung metastases were subjected to sternotomy with metastasectomy. Twenty-two pts with monostotic and 9 with metastatic OS were enrolled (M 15, F 16; median age 13 yrs, range 4-17; median FU 26 mos). rIL2 immuno-modulatory effects produced both in vitro and in vivo a striking transient increase of NK cells, as well as of NK and LAK activity. rIL2 side effects were always reversible and non-life-threatening. Surgical approach, histopathologic response to pre-operative treatment, and outcome were compared to those of the previous study of 48 pts treated without IT:

	1986-90 (no IT)	1991-95 (with IT)
monostotic	***37***	***22***
limb-sparing	20/37 (54%)	20/21 (95%)
necrosis ≥90%	17/36 (47%)	11/21 (52%)
DFS-SURV% (2 yrs)	54 - 78	70 - 80
metastatic	***11***	***9***
DFS-SURV% (2 yrs)	18 - 36	38 - 58

These data suggest that the addition of IT to conventional therapy may ameliorate the outcome of OS pts, but a longer follow-up is needed to confirm these preliminary results. *Supported in part by AIRC grant.*

P-141

POST-SURGICAL ETOPOSIDE AND IFOSFAMIDE IN OSTEOGENIC SARCOMA.

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Thirteen patients (6-40yrs) with non metastatic osteogenic sarcoma and a poor histologic response to pre-surgical chemotherapy, received post-surgical Etoposide (VP-16) and Ifosfamide (IFF). Before the surgeries, which included 2 amputations and 10 limb salvages, all had received two courses (two weeks apart) of high dose Methotrexate (8-12 g/sq.m. on days 1 and 8) followed by Cisplatinum (40 mg/sq.m. on days 15-19), and Adriamycin (45mg/sq.m. on days 15 and 16). Postsurgically, all received six 5-days courses at three week intervals of VP-16 (100mg/sq.m.), IFF (1800mg/sq.m.) and Mesna (1800 mg/sq.m.). The protocol was well tolerated with only one case of minor reversible renal insufficiency, and without hearing loss, urea or Creatinine abnormalities, or emesis (Ondansetron was given). At present, 8 (62%) patients are in CR, 1 is alive with disease(including lung metastases), and 4 had died. The post-diagnostic follow-up has lasted 13-84 months (mean 40 mos) and the post-treatment surveillance for a minimum of 6 months (median time 31 mos). The results suggest a benefit to disease progression-free survival time which must be verified by larger, long-term studies.

P-142

VANISHING BONE DISEASE: TWO EXTREME ENDS OF THE SAME DISEASE?

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Since Jackson described the first case of "vanishing bone disease" and Gorham and Stout collected twenty-four similar cases, linking the disease with an angiomatosis of blood and/or lymphatic vessels, there has been a controversy and confusion as to what constitutes "vanishing bone disease".

Is the angiomatosis a cause or effect of the disease?
Is it purely a bone disease?
Is the soft tissue component an integral part of the disease?

We present two cases of vanishing bone disease currently being treated at our centre.

The first case is a boy who presented with recurrent neck pains at the age of four. A skeletal survey showed multiple osteolytic lesions throughout the body. A biopsy of one of these lesions confirmed the diagnosis of vanishing bone disease. Management included a neck collar and oral etoposide.

The second case is a boy who presented age thirteen with an extensive lymphangioma involving the whole of his right leg. Xray of his bones were normal at that time. However a month later he sustained a pathological fracture and skeletal survey now showed multiple osteolytic lesions. Biopsy confirmed vanishing bone disease. His disease progressed relentlessly despite drainage procedures, oral etoposide and radiotherapy. He eventually had an amputation of the affected limb. Unfortunately his disease progressed further with involvement of the spine and he was started on experimental bisphosphonate therapy.

We would like to propose the idea that vanishing bone disease is a spectrum, one end of which consist of purely bony disease and the other purely soft tissue disease. These two cases illustrate this point.

P-143

THE ACETATE OF METHILPREDNISOLONE IN MICROCRYSTALS TOPICAL EFFECT IN THE ANEURISMATIC CYST OF THE BONE.

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The treatment with steroid infiltrations in unicameral bone cysts was first described, in 1974, from Scaglietti, but his efficiency in case of aneurismal bone cyst (A.B.C.) was often questioned. The Authors present some clinical reports of A.B.C. healed or improved by this therapy.
MATERIALS: Three patients, aged 11, 13 and 14 years, suffering from A.B.C. of the clavicle (recurred after resection), of the ileo and ischiopubic branch and of the calcaneus respectively, were threated with infiltrations. The diagnosis was always clinical, radiographic and histological. The infiltration of the lesion was performed under general anestesia and radioscopy; the dose of methylprednisolone varied from 80 to 120 mg. according to the size of A.B.A. In some cases 1 cc. od Iopamiro was added. Infiltration were repeated every 3 months, at least for 1 year.
RESULTS: After period ranging from 15 to 22 months, all the lesions healed or did not present clinical and radiographical features of recurrence.
CONCLUSIONS: Without refusing the role of surgery or other classical treatments (such as radiotherapy), on the base of their experience, the Authors conclude that infiltrations with methylprednisolone acetate in microcrystal proved to be effective in the therapy of A.B.C. and suitable for preparing patological bone for later surgery. They suggest this therapy when the seat of the lesion (such as in the pelvis) is presenting troubles in the surgical approach, or when traditional surgery has failed.

P-144

BIOCHEMICAL , INTRAOSSEOUS PRESSURE CHANGES AND TREATMENT OF THE BONE CYSTS.

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The fourty-two cases with solitary bone cysts the biochemical changes and intraosseous pressure changes were investigated . For each of the case pH, the partial pressures of oxygen and carbon dioxide, trace elements, alkaline and asid phosphates of venous blood and internal cyst fluid were measured. Also these measurements repeated after the withdrawn and 4 months later. We compared each of the cyst internal pressure measured before the metil-predniselone-acetate injection and after 4 months treatment. We used an elektromonometer,stop-cock,channel and bone marrow needle. Entering the cyst cavity was done under the CT. We demonstrated that the fluid of intracystic acid - alkaline phosphatase enzymes was increased very clearly and intracystic pO_2 and pCO_2 data was decreased than the venous blood.

The response of the cysts to the methylprednisolene acetate injection was searched. 25 patients were treated once: the remainder required second or third enjections. Twenty-eight lesions resolved completely, while 11 healed sufficiently to elininate the risk of pathologic fracture, and to permit physical activities without restrictions.

In all cases which have high alkaline and acid phosphatase level it is found that the bone cysts are active. In our analyses the intraossous pressure was high in active bone cysts but the inactive bone cysts the rise of this intraosous pressure was not very definite. The knowledge about the intra cysts fluid pressure and biochemical structure will give us more opinion on its etiology as its structure.

P-145

FAVOURABLE EFFECT OF CALCITONIN THERAPY IN CENTRAL GIANT CELL GRANULOMA OF THE MANDIBLE

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Central giant cell granuloma mostly occurs in the second decade of life; most common site is the mandible, followed by the maxilla. The lesions are non-encapsulated osteoclastic granulomas. Osteoid and new bone are noted between the granulomatous areas, although the lesions are not considered to be reparative. Curettage results in a recurrence rate of 25% and often leads to deformations and loss of teeth. Recently the favourable effect of calcitonin was reported by Harris (Br J Max Surg 1993,31,89-94).
Case: An 11 year old boy presented with a multicystic tumour in the right mandible expanding to the left side. The tumour (4x 3.5 cm) had displaced several (developing) teeth. At biopsy a central giant cell granuloma was diagnosed. Hyperparathyroidism was excluded. The patient was treated for 1 year with daily 60 U calcitonin (Cibalgin (R)) subcutaneously. The lesion showed clinically and radiologically (not yet complete) involution and the teeth attained a normal stability and eruption pattern. Side effects were minimal, i.e. short nausea at the moment of injection. Blood Ca, Ph and parathyroid levels stayed normal.
We conclude that calcitonin therapy should be the first option in treating giant cell granuloma. Regression takes a prolonged time. In case complete remission is not obtained, calcitonin treatment will probably reduce the extent of curettage and deformity.

P-146

ANEURISMAL BONE CYST . EMBOLOTHERAPY.

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Aneurysmal bone cysts (ABC) are benign, locally agressive vascular tumours of bone occurring in children and adolescents. ABC is an uncommon bone lesion representing 1% of all bone tumours. ABC is caused by circulatory disturbance. Progression may be very rapid and the cyst may reach a considerable size and produce clinical symptoms by compression of adjacent structures or as a result of pathologic fracture. ABC exist in association with some other type of bone lesion in approximately 30% of all cases. The most appropiate method of treatment is under controversy. Surgical approach with bone curetage is followed by a high rate of tumour recurrence. Radiotherapy may be effective but sarcomatous change has been described in some cases. Sponteaneous healing has been described. Indications for embolization of bascular bone tumours include decreasing the blood supply to limit blood loss at surgery or definitive therapy when surgery is not indicated. A 10 year old girl was referred for investigation of a painless mass within the pelvis. Plain radiograph showed large, expansile tumor placed in the superior pubic ramus with lytic appearance and thin cortical shell. CT and MR imaging add important diagnostic information.Diagnosis after surgical biopsy was ABC. After byopsy, clinical and radiological follow up was performed but pelvic mass increased its size and plain radiograph showed lesion progression. Angiography and posterior embolization of right accesory obturador artery with co-axial catheter and polyvinyl alcohol particles plus micro coils were performed. The cyst then showed progressive ossification and clinic symptoms disappeared. Spinal and pelvic lesion are a good indication for embolization in selected patients.

P-147

IN VITRO DRUG RESISTANCE AND PROLIFERATION MARKERS (Ki-67 AND PCNA) IN CENTRAL PRIMITIVE NEUROECTODERMAL TUMORS (PNET) IN CHILDHOOD.

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The aim of the study is to investigate the possible relationship between in vitro drug resistance for cisplatin (CDDP), etoposide (VP16), vincristine (VCR) and immunohistochemic proliferation markers (Ki-67 and PCNA) in PNET. Fifteen PNET samples (9 medulloblastoma, 6 supratentorial) from 14 patiens were tested. Drug resistance was measured by rapid automated MTT assay. In vitro drug concentration ranged from 0,09-100 µg/ml for CDDP 0,243-250 µg/ml for VP16 and 6,25-200 µg/ml for VCR. The results (expressed as drug concentrations lethal to 50% of tumor cells = LC50) were available for CDDP in 9 samples: median LC50 7 µg/ml; for VP16 in 10 samples median LC50 62.5 µg/ml and VCR in 11 samples median LC50 55.4 µg/ml. Ki-67 and PCNA were studied on paraffin embedded sections with a scoring scale for positive cells: 0 = 0%, I = 1-10%, II = 11-30%, III = 31-70% and IV > 70%. For Ki-67 the scores showed 2 samples with I, 4 with II, 8 with III and 1 with IV. For PCNA the scores showed 3 samples with II, 5 with III and 7 with IV. Linear regression analysis revealed an inverse correlation between sensitivity to VP16 and expression of Ki-67 with r = -0.7 and p value 0.034. In conclusion the preliminary results show that tumor samples with a high score on Ki-67 are more sensitive to VP16 in vitro. Investigation of a larger study population and possible relation with clinical outcome is in ongoing.

P-148

INTRAVENTRICULAR MAFOSFAMIDE IN PEDIATRIC BRAIN TUMOR PATIENTS WITH MENINGEAL DISSEMINATION

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Because of the limited penetration of systemically administered cytotoxic drugs across the blood brain barrier and the paucity of available agents for intrathecal therapy treatment for patients with tumor dissemination to the leptomeniges is generally unsatisfactory. Cyclophosphamide is an active drug in the treatment of a wide range of human malignancies including pediatric brain tumors . However, cyclophophamide is a prodrug that must be converted by hepatic enzymes to its active metabolites before it can express an antitumor effect. Mafosfamide is a preactivated derivative of cyclophosphamide, which does not require hepatic activation and thus can be utilized for regional therapy. Between May 1994 and January 1996, 12 patients 2 to 19 (median 12) years old with various poor prognosis malignant brain tumors were treated with intraventricular mafosfamide via an indwelling subcutaneous reservoir. Most patients had recurrent tumors and 10 patients had a positive cerebrospinal fluid (CSF) cytology and/or leptomenigeal metastases identified by MRI. The patients received mafosfamide at a dose of 20 mg weekly or biweekly until remission was achieved and every 3 to 4 weeks thereafter for maintenance therapy over a period of 4 to 13 months. Except for transient headaches and nausea which occurred in some of the patients during and immediately after mafosfamide administration no toxicities were observed. Six of the 12 patients were evaluable for response by CSF cytology. Four patients had complete responses following induction therapy with mafosfamide and in two patients multiple punctures yielded negative as well as suspicious cytologies. In addition to mafosfamide all patients received systemic chemotherapy and two also craniospinal irradiation. Currently, six patients are in complete and five in partial remission and one patient died of progressive disease. No leptomeningeal metastases were detected by MRI in any of the patients at last follow-up and the CSF cytology was repetitively negative in all patients. We conclude that mafosfamide might be a promising drug for intrathecal administration. Further research will show whether mafosfamide, if given at initial treatment, may help to reduce the dose - and in selected cases obviate the need for craniospinal irradiation.

P-149

CHEMOTHERAPY IN LOW-GRADE ASTROCYTOMA MANAGEMENT

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From 1989 to 1995, 19 children affected with incompletely resectable low-grade astrocytoma were treated. Fifteen were newly diagnosed and 4 were relapses; 11 were male and 8 female, with ages ranging between 12 and 168 months (average 93 mos). Two had neurofibromatosis.

Tumor sites were: 6 brainstem, 5 optic pathways, 3 cerebellum, 2 hypothalamus, 1 hemispheric, 1 episellar and 1 thalamus.

All patients showed severe symptoms. Sixteen cases underwent partial resection or biopsy, and in 3 (optic pathways) surgery was not performed.

Chemotherapy (CHT) consisted of carboplatin (CBDCA) at 1,000 mg/m^2 associated with etoposide (E) at 300 mg/m^2, in one day every 3-4 weeks for the first series of 13 pts and for the first four courses. Dosages were then reduced. Instead, CBDCA was given at 600 mg/m^2 and etoposide at 200 mg/m^2 in the remaining 6 pts and for the subsequent courses in the former 13 patients. The responses as ascertained by CT and/or MRI after four courses of CHT were: 1 CR, 6 MRs, 8 SDs and 4 PDs; 12 cases (63%) showed clinical improvement after CHT. The patients received 4 to 12 courses (average: 5.7). No toxic deaths occurred. Myelosuppression was manageable. Local radiotherapy (40 Gy) was administered after CHT in 14 cases, but in 3 of these RT was delayed for two years. Five pts did not receive RT; one because RT was performed before the relapse, 3 (optic pathways site) because of SD, and 1 because of young age.

The overall survival was 58% after an average follow-up of 22.5 months (range: 5-86 mos). Four patients are alive and disease free whereas 7 are alive with stable disease. Eight pts died of disease (6 with brainstem, 1 with thalamic, and 1 with optic pathways tumor); all of these had receveid local RT.

Brainstem tumor still exhibits a very poor prognosis. Recently, we treated three newly diagnosed brainstem tumors with partial resection and CHT (CBDCA at 600 mg/mq and E at 200 mg/mq) for 2-4 courses, followed by radiosurgery. After 15 months, two children are alive with GPR and one died of disease progression 8 months after diagnosis.

Therefore, in unresectable symptomatic low-grade astrocytomas CHT with CBDCA associated with E can be used to postpone radiotherapy in young children and to avoid radiotherapy even in some cases, whereas in high-risk sites CHT can be used in association with radiosurgery.

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P-150

CYTOGENETIC FLUORESCENCE IN SITU HYBRIDIZATION AND MOLECULAR STUDIES IN A MEDULLOBLASTOMA WITH t(1;19)

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In cytogentic analysis of acute lymphoblastic leukemia (ALL) a chromosomal abnormality t(1;19) is associated with a poor prognosis. We report here the first observation of a t(1;19) in a case of childhood medulloblastoma and more widely in a cerebral tumor.

A 4 year girl presented with a posterior fossa medulloblastoma showed good prognosis factors after the initial removal and received the M-SFOP protocol. A supratentorial multifocal relapse occured on year post-surgery and 7 months after the end of treatment. Cytogenetic analysis and two-color fluorescence in situ hybridization (FISH) realized on cell cultures of fresh tumor samples showed the rearrangement between the chromosomes 1 and 19. Molecular studies of the rearrangement of the genes E2A and PBX1 who are involved in the ALL with t(1;19) showed that the transcrit E2A-PBX1 is not involved in the t(1;19) observed in our patient. Explanations possible are that the breakpoints of the t(1;19) are different in medulloblastoma and in ALL, or perhaps that different genes are involved in this rearrangement. We wonder if such a translocation could carry out poor prognosis in medulloblastomas as in ALL. Further studies are required to investigate if same genes are implicated in these two t(1;19).

P-151

PROGNOSIS AND TREATMENT OF BRAIN TUMOURS IN CHILDHOOD - A SINGLE INSTITUTION EXPERIENCE OF 16 YEARS

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Since 1979, 230 children with brain tumours were diagnosed in our Unit. 123 (53.4%) were boys and 107 (46.6%) girls. The age at diagnosis ranged from 6 mo to 15 yrs (mean age 7yrs±3,78). 102 (44%) children had infratentorial and 128 (56%) supratentorial tumour. Histology was available in 205 cases: 78 children had astrocytoma (38%), 35 children had medulloblastoma (17%), 18 children had mixed glioma (18.8%), 16 children had craniopharyngioma (7.8%), 15 children had ependymoma (17.32%), 10 children had konarioma (4.9%), 8 children had germinoma (3.9%) and 25 children had other tumours (11.2%). The patients were treated with surgical excision, chemotherapy, radiotherapy or combination of them. The therapheutic approach in each case was determined according to the age of the patient, the histological classification, the location and the stage of the tumour. The overall 5-year survival rate (method Keplan-Meier) was 61% (±3.9%) while the 10-year survival rate was 54% (±4.9%). In details the 5-year survival rate for the astrocytomas was 79% (mean survival time: 9 yrs), for the medulloblastomas 65.6% (mean survival time: 7 yrs), for the craniopharyngiomas 90% (mean survival time: 15 yrs). The ependymomas had very poor prognosis (mean survival time: 2yrs). Better survival was noted in cases of: 1) total resection of the tumour, 2) children >10 yrs of age compared to children <2 yrs. *In conclusion*, the prognosis of brain tumours in childhood is more favorable now, compared to the past decades and that seems to be attributed to the easier diagnosis and the improvement in the treatment methods.

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MINIMALLY INVASIVE PROCEDURES FOR THE KARYOTYPING OF SOLID TUMOURS.

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It has been considered that one of the draw backs of conventional karyotyping of solid tumours is that large surgically resected tumour specimens are required. We investigated with minimally invasive procedures, whether it is possible to obtain a good quality karyotype from tumour infiltrated material.

Thirteen children, with five different malignant tumours, had either a core needle biopsy of a tumour mass (4 tumours), an aspirate of either pleural fluid (1), ascites (1), CNS fluid (1) or bone marrow (6).
All specimens were cultured according to recognised cytogenetic methods followed by G-banding analysis.

From 12 out of 13 tumours a karyotype was obtained, all of which were abnormal (4 Wilms tumours, 5 neuroblastomas, 1 osteosarcoma, 1 Ewings sarcoma, 1 hepatocellular carcinoma). The single failure was from a needle biopsy (Wilms tumour). In all cases the result was reported within two weeks of sample collection. The karyotypes found included important diagnostic or prognostic information such as detection of double minutes and del(1p) in neuroblastomas, a t(11;22) translocation in a Ewings sarcoma (from the CNS fluid aspirate) and an abnormal karyotype in an osteosarcoma where bone marrow infiltration was not suspected.

We could demonstrate that clinically important karyotypic information can be obtained from specimens obtained by minimally invasive procedures. Therefore, cytogenetic analysis should be considered for all needle biopsies or aspirates where tumour infiltration is possible.

P-153

Combined Cell Sorting and FISH for detection of minimal disease in bone marrows of children with acute leukemia or solid tumor

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The ability to detect residual blasts and/or to evaluate minimal bone marrow (BM) metastasis is a major challenge in the treatment of neoplasias. Several techniques have been used to distinguish neoplastic from normal cells; a combination of different approachs seems to achieve the best results. Abnormal karyotypes have been frequently found in neoplasias. This approach permits an unambigous identification of neoplastic cells but with very low sensibility. To increase this we utilize FISH to identify known numerical chromosomal abnormalities in a population of cells previously sorted by peculiar phenotypic markers. BM of 6 children (3 ALL, 2 NB and 1 PNET) were studied. The cases were selected for having karyotypic abnormalities at diagnosis. BM immunophenotype analyzed with FACS, didn't shown any minimal disease in all the cases. In the solid tumors the bone biopsies were negative. For FISH analysis a satellite DNA probes were used and detected with the standard method. At least 500 cells were analyzed by two persons. As control we used a leukemic BM with trisomy 6 at different dilutions. After sorting the sensibility increased 10 times.
In the BM of the patients with NB MYCN amplification in some CD45-CD56+ cells was identified; one patient relapsed two months after. In all others cases no "neoplastic" cells were detected with this method. The children are well 2 to 6 months after.
Our preliminary results show that the combination of traditional cytogenetic, immunophenotyping, sorting and FISH technology could be useful to detect minimal disease in selected cases.

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EXTRACRANIAL TERATOMA: AN ITALIAN POLICENTRIC STUDY

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Cooperative Group AIEOP-ITALY.

Teratoma are benign tumors, however in some cases they can recur, with malignant histology. With the aim of upgrading the knowledge of Teratoma natural history, a policentric study was activated in Italy since January 1991 to December 1995; 15 Centers partecipated; 94 patients (pts.) were enrolled: 73 pts.(43 F, 30 M) had Mature Teratoma (T.) and 21 pts. (14 F, 7 M) had Immature T. (grade I: 1 pt., II: 12 pts., III: 8 pts.). Age of pts.: Mature T. 0-14 yrs. (median 14 mo.); Immature T. 0-14 yrs. (median 13 mo.). Site of the tumor: 1) Mature T.: s.c. 34 cases, testis 14, ovary 13, mediastinum 5, retroperitoneum 3, other:4 cases. 2) Immature T.: ovary 8 cases, s.c. 5, testis 4, other:4 cases.
Treatment: 1) Mature T.: surgery only. 2) Immature T.: testicular or grade I, all sites: surgery only; grade II-III, extratest.: surgery + chemotherapy (CT) (VBL, EDX,ACT-D).
Results: 1) Mature T.: resection was not complete in 12 out of 73 pts.: one of them had malignant recurrence; following CT (Carbo-VP16/IVA), the pt. is disease free for + 4 mo . The other 72 pts. remained disease free for 8 to 56 mo. (median 46 mo.). 2) Immature T.: one* pt. had biopsy, CT, surgery, CT; she had relapse 10 mo. after surgery. Complete resection was performed in 20 pts., at diagnosis; CT was not admistred in 4 pts. (out Protocol), and in 7 pts. (according to the Protocol). Relapse occurred in 4 of 17 pts. in Protocol and in 1 pt. out Protocol. Sixteen of 21 pts. remained disease free for 6 to 36 mo. (median 9 mo.).

Table: RELAPSE OF IMMATURE T.

Pt.	Site	Grade	CT	Histology at Relapse
1	ovary	III	yes	Malignant
2	ovary	III	yes	Immature T.
3	ovary	II	yes	Mature T.
4	retroper.	III	no	Immature (II relapse: Mature T.)
5*	s.c.	III	yes	Immature T.

Conclusions: for Mature T. surgical complete resection at diagnosis is mandatory; a long term follow-up is useful to detect recurrences. For Immature T. is not clear if more aggressive CT is necessary or not; a randomised study seems necessary.

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INTRACRANIAL EPENDYMOMA - A REVIEW OF 25 CASES.

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Method: Retrospective chart review of patients (pts) treated between 1979 - 92.
Results: 13 male and 12 female pts. Mean age at diagnosis was 4.6 yrs. 3 pts had supratentorial and 22 had infratentorial primaries. 12 pts underwent gross total excision and 13 had incomplete resection. All pts had apparently localised disease at presentation. 12 pts received local radiotherapy (XRT) alone (mean dose 51.7 Gy) while 13 pts received craniospinal XRT (mean dose 31.7 Gy to neuroaxis & 50.7 Gy to primary). 13 pts received additional chemotherapy.
13 pts relapsed (10 after craniospinal XRT & 3 after local XRT alone). All pts relapsed at the primary site (2 pts relapsed both in spine and primary). All these patients are dead from disease. In relapsed pts the mean duration from diagnosis to initiation of therapy was 39 days & in relapse free pts this was 24 days.
12 pts are alive and disease free after mean follow up of 7.7 yrs (range 2.8 - 16.5 yrs). The 5 year survival was 60%. Only 2 survivors have severe developmental delay & both had craniospinal XRT. Chemotherapy did not appear to improve outcome. Complete tumour resection appeared to reduce the risk of local relapse.
Conclusions: Craniospinal XRT is of no benefit and increases morbidity.
Delay in initiation of XRT may be associated with a worse prognosis.

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CHEMOTHERAPY TREATMENT FOR PAEDIATRIC PRIMARY CENTRAL NERVOUS SYSTEM (CNS) GERM CELL TUMORS

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From Jan 1990 to Dec 1995, 7 patients (6 males, 1 female) were treated in the Paediatric department. The mean age at diagnosis was 10.9 years (range 2.9 - 14.3 years). Two suffered from Downs syndrome. There were 5 germinomas, 1 yolk sac tumor and 1 malignant mixed teratoma. Raised serum and cerebrospinal fluid alpha fetoprotein was noted in 2 patients. No spinal cord metastasis was noted. Surgical biopsy (in 1 patient) and debulking (in 6 patients) was performed at the time of diagnosis. 3 patients had radiotherapy as initial treatment (4500 cGy to tumor region). As residual tumor was evident on imaging in these 3 patients, they received chemotherapy with blecomycin, etoposide and cisplatinum (BEP). The other 4 patients had chemotherapy (3 with BEP, 1 with BEP + vincristine + methotrexate) initially. All patients showed good tumor shrinkage to chemotherapy. Patient with malignant mixed teratoma required radiotherapy subsequently because of residual mass on MRI studies. One Downs syndrome died of pneumonia complicating chemotherapy who still had a residual mass on MRI studies. 2 patients treated with chemotherapy alone had complete disappearance of the tumors. Six patients survived without evidence of recurrence. Median follow up period was 24 months (range 6 - 71 months). In conclusion, CNS germ cell tumors appeared to respond favourably to BEP chemotherapy ± radiotherapy. The effectiveness of chemotherapy alone and long term complications will require a multicenter clinical trial for evaluation.

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TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION (TACE) FOR TREATMENT OF HEPATOBLASTOMA IN INFANTS

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The prognosis of hepatoblastoma is very poor unless the tumor is resected, so that various chemotherapy have been developed to decrease the size of this tumor. Recently transcatheter arterial chemoembolization (TACE) has been well accepted as an effective and safe method for treatment of unresectable adult hepatoma. We applied this procedure to hepatoblastoma in infants and observed a favorable response.
PATIENTS AND METHODS: TACE was performed in five huge hepatoblastoma cases which occupied more than one hepatic lobe. An intraarterial catheter was inserted into right or left hepatic artery, which was the main feeding artery of the tumor. Injection of THP-adriamycin (20-30mg/m^2) dispersed in Lipiodol and cisplatinum(40-60mg/m^2) followed by gelfoam pieces were performed. Effects of TACE were evaluated by shrinkage on imaging examinations, decrease in alpha-fetoprotein(AFP) level and pathological findings of the surgical specimens.
RESULTS: A marked reduction in tumor size and decrease of AFP level occurred one month after the treatment. Mean reduction rates of the tumor size and AFP level were 37% and 93% respectively. Toxicities to anti-tumor drugs were not noted except for moderate fever for a few weeks. Resection of reduced size tumors were performed in safety and every cases are alive except one who died of lung and brain metastasis 3 years after operation. Pathological examination showed massive necrosis in the surgical specimens. Mean percentage of the necrotic area was 81%.
CONCLUSION: TACE should be a very effective, safe and useful method in the initial treatment of huge hepatoblastoma.

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IDENTIFICATION OF ENRICHED SEQUENCES FROM A SUBTRACTIVE-HYBRIDIZATION PROCEDURE IN REGENERATING LIVER, ASSOCIATED WITH HEPATOBLASTOMA

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Introduction The initiation and termination of liver regeneration is strictly regulated, and controlled by a set of factors/genes that is only partly characterized. Failure in this regulation could lead to carcinogenesis, and carcinogenesis could on the other hand also enhance itself by the production of hepatotrophic factors by the tumor.
Aim Identification of regeneration related gene products, and assessment of their expression in hepatoblastoma.
Methods cDNA was prepared from rat liver under regenerating conditions, induced by partial (70%) hepatectomy and isolation of mRNA after 12 and 32 hours. Simultaneously, cDNA was prepared from a non-regenerating liver, a sham-operated animal after identical time points, and from a rat liver with a chemically (diethylnitrosamine) induced hepatocellular carcinoma. Elimination of the common gene products and the enrichment of up-regulated cDNA's (proliferation stimulation factors) and down-regulated cDNA's (inhibitory factors) was successfully done by a cDNA subtraction/hybridization technique. The resulting up- and down-regulated cDNA populations, obtained with the method described above, were analyzed by a differential display technique. The cDNA fragments were compared on a polyacrylamide gel, and the differences between the populations were identified. Subsequently, these fragments were used to isolate the complete cDNA from a liver cDNA library, and were characterized and analyzed.
Results Over 50 up- and down regulated cDNA's from regenerating liver, and diethylnitrosamine induced hepatocellular carcinoma were isolated, and characterized by nucleotide sequence analysis. The majority were well characterized regeneration, transcription oncogenes and tumor-associated geneproducts. Novel genes were analyzed on Northern Blot. So far, several RNA probes were constructed and used in in situ hybridization experiments. Expression of these probes in hepatoblastoma tissue and the subtractive hybridization display method will be presented.

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THE CHILDHOOD HEPATOBLASTOMA (HB) AND HEPATOCELLULAR CARCINOMA (HCC) TREATED BY PRIMARY SURGERY FOLLOWING CISPLATIN (PLA) AND DOXORUBICIN (DO)

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Between 1/90 and 12/95, 13 patients (pts) with liver tumors (11 HB and 2 HCC) involving one liver lobe were treated according to scheme: complete resection following chemotherapy PLADO (cisplatin 80mg/m^2 i.v. over 24 hours, then doxorubicin 60mg/m^2 i.v. over 48 hours). Median age at diagnosis was 1y 11m (range 2m-6y 9m) and 15y 6m for HB and HCC, respectively. Male to female ratio 1,7:1 and 1:1, respectively. Serum alpha-fetoprotein (AFP) level was elevated in 90% HB. There were no post-surgical complications (surgery included complete resection in 12 pts and subtotal resection in 1 child). Extend of resection of primary tumor has been documented using sonography , CT and FP level. Chemotherapy involving 2-6 courses PLADO in 12 pts and monotherapy DO in 1 child (HB). The toxicity of chemotherapy has been mild.
In January 1996 ten (90,9%) HB pts and two (100%) HCC are in complete response (CR) and one (9,1%) HB died due to sepsis. Conclusion: 1) primary complete resection of liver tumors is possible and recommended, 2) tolerance of the post-operative chemotherapy PLADO is good and 3) the amount of courses of chemotherapy is not exact determined and will be discussed.

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PHARMACOKINETIC EVALUATION OF INTRAHEPATIC ARTERIAL INFUSION THERAPY OF THP-ADR AND CDDP IN PATIENTS WITH HEPATOBLASTOMA

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The selective intraarterial infusion therapy for hepatoblastoma is still controversial. We have treated 3 cases of hepatoblastoma with intrahepatic arterial infusion of THP-adriamycin (ADR)(60 mg/m2) followed by the infusion of CDDP (90 mg/m2) over 20 minutes. Neither severe hepatic toxicity nor bone marrow suppression were encountered with this treatment. Pharmacokinetic studies revealed that maximum plasma concentrations of THP-ADR ranged from 0.03 μg/ml to 1.71 μg/ml. The area under the plasma concentration x time curve (AUC) value ranged from 3.89 μg x min /ml to 65.66 μg x min /ml. In contrast, when THP-ADR (60 mg/m2) over 48 hours was infused intravenously, AUC value ranged from 270 μg x min /ml to 1924 μg x min /ml. AUC value were significantly lower in intraarterial infusion group than in intravenous infusion group. These results suggest that the selective intraarterial infusion of THP-ADR could be performed without any severe organ toxicity. Moreover lower systemic AUC values and probably higher THP-ADR concentrations in tumor tissue itself would be expected.

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GENE EXPRESSION PATTERNS IN HEPATOBLASTOMA

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Introduction Hepatoblastoma is the most common hepatic tumour in children. Although different types of hepatoblastoma can be distinguished, the phenotypic properties of these cells are poorly characterized. For that reason we stained a number of specimens with antibodies that define structural compartments of the liver.
Methods Carbamoylphosphate synthetase (CPS) and glutamine synthetase (GS) were used as marker proteins for the periportal and pericentral regions of the liver, respectively. Cytokeratin19 (CK19) was used to mark the bile ducts, while α-smooth muscle actin (αSMA) and desmin were used to identify Ito cells. None of the tumours were pretreated with cytostatic drugs.
Results All tumours were characterized by extensive septa of fibrotic tissue, which surrounded fields of hepatoma cells. The least differentiated type of tumours showed large fields of undifferentiated cells that did not stain with any of the hepatocyte markers, but which still contained CPS-positive hepatoma cells at its junction with the fibrous tissue. This fibrous tissue contained numerous CK19-positive bile-duct like cells. In the more differentiated type of hepatoblastoma, large fields of CPS-positive hepatoma-cells were found. A tract of GS-positive hepatoma cells was usually found at the periphery of the nodule, while isolated GS-positive cells were found dispersed throughout the nodules with a distribution pattern that paralleled that of αSMA. CK19-positive cells were found between the strand of GS-positive cells surrounding the nodules and the fibrous tissue. Unexpectedly therefore, the normal polarity of cell phenotype in the liver, viz. fibrous tissue of the portal tract > bile ducts > periportal (CPS+) > pericentral (GS+) cells was changed with the GS+ phenotype inserted between the CPS+ and the CK19+ cells. This observation appears to reflect an abnormal induction pathway in hepatoma cells.

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METASTASIZING CLEAR CELL SARCOMA OF THE KIDNEY

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Introduction: Clear cell sarcoma of the kidney (CCSK) is a rare and highly malignant tumor, comprising 4-6% of primary childhood renal tumors. Here we describe a 4 year old patient with CCSK who developed a lung metastasis 3.5 years after the primary and consequently a brain metastasis 4 years after the primary CCSK.
Case report: A four year old girl was admitted to our hospital, with a large tumor of the left kidney (13x10x11cm), without signs of metastases. On the presumptive diagnosis of Wilms tumor, preoperative chemotherapy according to the SIOP-9 protocol (ActD/VCR) was initiated. After 4 weeks of therapy, without any signs of reduction, a left radical tumor-nephrectomy was performed. Pathologic examination revealed the diagnosis CCSK, totally removed, stage I. Chemotherapy was changed to VCR/Dox/Cyclo for 8 months. A routine X-thorax 42 months after diagnosis, revealed a single pleural metastasis. CT-scan of the brain and a bone-scan didn't show any other metastases. A complete metastasectomy was performed and histology confirmed a metastasis of the primary CCSK. No further therapy was administered. Six months later, without any complaints, a CT-scan of the head revealed a right parietal lobe tumor. A parieto-occipital craniotomy was performed and gross total removal was achieved. Again the histology was identical to the primary tumor. Postoperative radiotherapy was given, followed by chemotherapy (Carbo/VP16). At the moment the patient is at the end of her chemotherapy and is doing well.
Literature review: A review of the literature since 1976 reveals more than 40 manuscripts of CCSK, with only 4 focussing on mainly bone metastases. To our knowledge no cases of brain metastases after a follow-up of 4 years are reported.
Conclusions: Although chemotherapeutic advances have improved the outlook for children with CCSK, the disease may ultimately be fatal from local recurrences and systemic metastases, especially to bone and lung. Bone metastases, the reason of the bad prognosis of this so-called "bone metastasing tumor of infancy" seem to develop early. Patients with a CCSK need a very long follow-up with a careful regimen of frequent investigations of all the possible areas of relapses, including the brain.

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NEUROFIBROMATOSIS TYPE 1 GENE EXPRESSION IN PHEOCHROMOCYTOMAS AND PRIMARY BRAIN TUMORS

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Abstract:
Neurofibromin, the protein encoded by one of the most important familial cancer genes, the NF1 gene, includes the NF1 GAP-related domain (NF1 GRD), that downregulates the activity of p21 ras and could be responsible for the tumor suppressor function of the gene. Neurofibromatosis type 1 is characterized by an increased incidence of malignancies, prevalently of neuroectodermal origin. Absence of neurofibromin has been documented in tumors, both sporadic and derived from NF1 patients, but a number of studies have failed to identify structural alterations of the NF1 GRD that could account for loss of its putative tumor suppressor function. Two NF1 splice variants, neurofibromin I and II are generated by the insertion of 21 amino acids within the NF1 GRD, the study of these isoforms may help to understand the role of the NF1 gene in tumorigenesis. The NF1 expression pattern has been studied in 26 samples of pheochromocytoma, a tumor common to other cancer family syndromes like VHL, tuberus sclerosis and Sturge-Weber disease, in the normal tissue counterpart adrenal medulla, and in 4 primary brain tumors. The study has been conducted by reverse transcription of NF1 messenger RNA and amplification of overlapping fragments representing the whole GRD sequence (RT-PCR). Equal expression of neurofibromin I and II has been consistently detected in normal adrenal medulla while a different pattern, characterized by the prevalence of neurofibromin I transcript, has been observed in 23/26 pheochromocytomas; three samples of our series, two of which malignant, exhibit either equal expression of the two isoforms or a definite prevalence of neurofibromin II. Unequal expression of the two transcripts has been observed in the brain tumors examined, but the data need to be confirmed on a larger sample. We never detected absence or reduced expression of neurofibromin in our tumor samples. We think differences in neurofibromin expression pattern, not only quantitative alterations of the message, might be important in determining the role of the NF1 gene as part of the mechanisms controlling cell growth and differentiation.

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DETECTION OF SPECIFIC CHROMOSOMAL TRANSLOCATIONS IN PEDIATRIC SOFT TISSUE SARCOMAS BY RT-PCR.

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Cytogenetic studies have identified specific chromosomal alterations in some pediatric soft tissue tumors. The specific reciprocal traslocations t(2;13)(q35;q14), or the rarer variant t(1;13)(p36;q14) have been reported in the alveolar rhabdomiosarcoma (A-RMS), whereas the t(11;22)(q24;q12) has been described in the PPNET-Ewing group of tumors. Recently the development of molecular techniquess have greatly improved the sensitivity in the detection of genetic abnormalities as compared to standard cytogenetic analysis.
We studied the specific traslocations t(2;13), t(1;13) and t(11;22) in a series of 49 soft tissue sarcomas of childhood by RT-PCR, using specific primers for the following chimeric transcripts: PAX3-FKHR, PAX7-FKHR, originated from the t(2;13) and t(1;13), respectively, and EWS-FLI1 derived from the t(11;22).
In our series 43 patients had a RMS: 24 alveolar, 15 embryonal and 1 leiomyomatosus; 3 were RMS without other specifications. Among the other non-rhabdo sarcomas 4 were PPNET, 1 Malignant Peripheral Nerve Sheath Tumor and 1 Leyomiosarcoma. Analysis of the 49 samples revealed the chimeric transcript originated from the t(2;13)(q35;q14) among the A-RMS (16/24) and in one case of E-RMS (1/15). The rarer variant PAX7-FKHR was identified in 2 cases of A-RMS (2/24). Thus, 18/24 A-RMS expressed one of the two expected fusion transcripts, while they were not found in any of the non-RMS sarcomas. The EWS-FLI1 product was detected in 3/4 PPNET. All tumors studied expressed only one chimeric transcript.
Our study confirms the specific association of the t(2;13) and t(1;13) with the alveolar subtype of RMS. The absence of chimeric products in 6 alveolar RMS could depend on negligible expression. Alternatively, the presence of t(2;13), or the rarer variant t(1;13), could be limited to a subgroup of alveolar RMS.
We conclude that RT-PCR is a useful molecular technique for the diagnosis and staging work-up of patients with soft tissue sarcomas. In order to evaluate the efficacy of this approach in detecting minimal disease in patients with bone marrow involvement, we have initiated a multicentric prospective molecular study. *Emanuela Frascella is recipient of a fellowship from AIRC.*

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DELETION MAPPING OF MEDULLOBLASTOMA ON CHROMOSOME 17p

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Medulloblastoma is the most common malignant tumour of the central nervous system. Cytogenetic analysis of these tumours has demonstrated alterations of chromosome 17, in particular isochromosome 17q, as the most frequent chromosomal abnormality. Since the consistent loss of a specific chromosomal region indicates a tumour related gene, we performed a deletion mapping of a medulloblastoma panel using new microsatellite markers from a high resolution genetic map of chromosome 17p (1). A fine mapping of this region with highly polymorphic markers has not been published before.
Methods: Blood and tumour pairs of consecutive patients operated upon for pathologically confirmed medulloblastoma were collected. We used a set of 11 PCR-able microsatellite markers, all located in 17p.
Result: We confirmed indirect evidence of a putative tumour suppressor gene on chromosome 17p. Loss of distal chromosome 17p13.3 sequences were revealed in 9 of 16 (56%) patients.
The smallest region of overlap defines a chromosomal region which harbours the tumour-gene and is a prerequisite for the isolation of a candidate-gene. We identified two patients with a microdeletion, which narrows down the region to about 1 Mb. These data will be important to isolate YACs and genes within this defined region.

Literature:
1. A strategy for constructing high-resolution genetic maps of the human genome: A genetic map of chromosome 17p, ordered with meiotic breakpoint-mapping panels. Am J Hum Gent,56:484-499, 1995

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LOSS OF HETEROZYGOSITY AT 17p IN CNS EMBRYONAL TUMORS.

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Molecular events are being used as markers of specific tumors or as milestones of tumor progression. But most of the molecular events in the neoplastic transformation remain unknow. Cytogenetic analysis of CNS embryonal tumors has demostrated isochromosome 17q, as the most frequent chromosomal abnormality. Molecular studies by RFLP's analysis has confirmed loss of genetic information on 17p. The common area of loss has been restricted to 17p11.2 to 17 pter. This region contains the p53, gene,a role for this gene in PNET has been hypothesized. However, mutations in p53 gene are very rare in these tumors. This strongly suggests that another, as yet unidentified gene on 17p may be involved in these tumors.

We have performed a search of both loss of heterozigosity on 17p and p53 mutations (exons 5-8) by microsatellite markers and SSCP analysis respectively, on 20 childhood CNS tumours of distinct histologic types. We have detected loss of heterozigosity in 5 cases all of them embryonal tumors. Four of them did not imply the p53 locus while the other one presented a mutation in exon 7, not yet sequenced. The area of common loss of heterozigosity in our cases includes markers D17S926 and D17S938, and currently we are working with new markers in this interval to further delimit the region containing a new supressor gene.

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ESTABLISHMENT OF A NEUROBLASTOMA CELL LINE BEARING AN N-*myc* GENE AMPLIFIED AND REARRANGED

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It is well known that advanced neuroblastoma shows the N-*myc* amplification and it is associated with the poor prognosis. The N-*myc* activation is believed to be achieved principally through the increase in it's copy number. In the present study, we established a cell line bearing an N-*myc* gene not only amplified but also rearranged. MATERIALS & METHODS: Cells were cultivated from a stage III neuroblastoma in a 26-m-o-girl. The new cell line, NB(TU)1, was morphologically confirmed as neuroblastoma. The molecular analyses were carried out by Southern and Northern hybridization and also by comparative genomic hybridization (CGH). RESULTS & DISCUSSION: Cells had the tendency to project neurites spontaneously, indicating that this cell line can be a highly differentiated one. Neurite formation was enhanced by the treatment of db-cAMP. Northern hybridization showed the N-*myc* mRNA at a comparable level to other cell lines with N-*myc* amplification. Southern hybridization using a probe for exon 2 of N-*myc* detected 2-kb band at the intensity of 30-fold. On the other hand, that using 3-kb *Eco*RI-fragment containing exon 1 showed a 9-kb aberrant band in addition to a 3-kb ordinary band. The 9-kb band was also detected in xenograft tissues transplanted in nude mice but not in patient's peripheral leukocytes. CGH analysis revealed that amplified DNA fragments came solely from the original locus of N-*myc*, 2p24.1, suggesting an intrachromosomal rearrangement. CONCLUSION: To our knowledge, this is the first report of the rearrangement within the exon 1 or 5'-untranscribed region. As the 5'-region has been considered to be associated with transcriptional control either positively or negatively, the relationship between the rearrangement and the N-*myc* expression remains to be clarified. NB(TU)1 would be a useful tool for such studies.

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EVIDENCE OF GENETICAL CHANGES IN THE SHORT ARM OF CHROMOSOME 1 OCCURRING DURING PROGRESSION IN NEUROBLASTOMA

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Neuroblastoma is characterised by genetical abnormalities in the short arm of chromosome 1. Presently it is unclear whether changes in 1p are a primary event in tumorigenesis or occur later during tumour progression. We present here evidence that genetical changes in 1p can occur during progression. Pat. 1, male, was diagnosed with an adrenal neuroblastoma stage IV at the age of 5 months. Metastases were present in the lymph nodes, bone and bone marrow. Ferritin (159 μg/L) and LDH (970 u/L) were increased. Molecular genetical studies revealed no N-myc amplification and no loss of region 1p34-1p36.3. Cytogenetical studies on the bone marrow showed an abnormal clone with no 1p abnormalities. Tumour progression occurred within 15 months after MIBG treatment and during chemotherapy. Cytogenetical studies on the bone marrow revealed the original abnormal cell clone with additional abnormalities including an interstitial deletion 1p32p34. Pat. 2, female, was diagnosed with a stage IV adrenal tumour at the age of 3 9/12 years, with bone marrow and bone metastases. Ferritin level was normal (58 μg/L), whilst LDH was increased (797 u/L). The tumour had no N-myc amplification and showed LOH for region 1p36.3. Cytogenetical studies showed an abnormal clone with no 1p abnormalities. Tumour recurrence occurred after 15 months. A bone marrow sample with 10% infiltration contained metaphases with the same abnormal clone as at diagnosis but with extra abnormalities including a 1p+ chromosome (add(1)(p32)). Molecular and cytogenetical studies on two children with recurring neuroblastoma have demonstrated that genetical changes in the short arm of chromosome 1 can occur during tumour progression.

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9-*CIS* RETINOIC ACID IS A BETTER RETINOID FOR THE DIFFERENTIATION THERAPY OF ADVANCED NEUROBLASTOMA

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Clinical trials of both 13-*cis* and all-*trans* retinoic acid (RA) in patients with advanced neuroblastoma have demonstrated limited success in the maintenance of sustained remission. This may be due, at least in part, to rapid clearance from the plasma and autometabolism of the all-*trans* isomer. 9-*cis* RA has been reported to have more favourable pharmacokinetics and is presently being evaluated in a number of adult malignancies. We have investigated the *in vitro* effects of 9-*cis* RA on the SH SY 5Y neuroblastoma cell line and show that 9-*cis* RA is 10 fold better at producing sustained morphological differentiation and inhibiting the proliferation of SH SY 5Y cells than other RA isomers. RA is thought to promote neuroblastoma differentiation by inducing gene expression. Retinoic acid receptor-ß (RAR-ß), which may be prognostically significant in neuroblastoma, is induced by all-*trans* RA in neuroblastoma cells. We have therefore studied the effects of 9-*cis* RA on RAR-ß, and another gene, cellular retinoic acid binding protein II (CRABP II), which is also induced by RA. As with morphological differentiation, high concentrations of 9-*cis* RA are more effective than other isomeric forms at inducing gene expression. In contrast to all-*trans*, 9-*cis* RA does not bind to CRABPs and therefore may be more stable *in vivo*. These studies indicate that 9-*cis* RA may have greater therapeutic potential for the differentiation of neuroblastoma.

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SYNERGISTIC EFFECTS OF SODIUM PHENYLACETATE AND CONVENTIONAL CHEMOTHERAPEUTIC DRUGS IN THE TREATMENT OF NEUROBLASTOMA CELLS

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Sodium phenylacetate (NaPA) is a naturally ocurring plasma component that suppresses the proliferation and stimulates the differentiation of neuroblastoma cells by inhibiting the mevalonate pathway at concentrations that have been achieved in adults and children without significant side effects. Mevalonate is the precusor of a number of different products potentially required for the growth of malignant cells, including the prenylated oncoprotein ras. The effects of combinations of NaPA and other clinically approved chemotherapeutic drugs such as Doxorubicin (DXR), Vincristin (VCR), arabinosylfuranosylcytosine (ARA-C) and all-trans retinoic acid (RA) on neuroblastoma cells (UKF-NB-3 and UKF-NB-2) were studied in vitro. DXR and VCR demonstrated antagonistic and additive effects. In contrast ARA-C and RA exhibited highly synergistic effects. ARA-C and NaPA showed a combination index ranging from 0.83 to 0.95, whereas RA and NaPA presented a combination index ranging from 0.87 to 0.1. In a seperate test we were able to proof increased expression of RA-receptor expression upon NaPA treatment of neuroblastoma cells. This might explain the extreme synergistic effect on neuroblastoma cell differentiation induction and is therefore an interesting combination for differentiation therapy of neuroblastoma patients.

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DEVELOPMENT OF POLYCLONAL ANTIBODY AGAINST SYNTHETIC PEPTIDE FROM N-MYC ONCOPROTEIN: COMPARISON BETWEEN HEMOCYANIN CONJUGATION AND MULTIPLE ANTIGEN PEPTIDE METHODS

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The importance of determining N-myc oncoprotein rather than genomic N-myc amplification in neuroblastoma has been emphasized because of the discrepancies between prognosis and N-myc amplification observed in some neuroblastoma cell lines and clinical Stage IV patients. In order to develop an ELISA for the N-myc oncoprotein, an effort was made to raise antibodies specific for N-Myc. N-Myc specific peptides, HGRGP PTAGS TAQSP G (No.136-151 of N-Myc) and GVAPP RPGGR QTSGG DH (No.223-239) were synthesized and injected weekly in the subcutaneous tissue of Japanese rabbits in conjugation with either hemocyanin or lysin core (multiple antigen peptide method) plus adjuvant. Synthesized peptides conjugated to lysin core (MAP method) raised much more potent antibodies than those peptides conjugated to hemocyanin. Both crude antibody and IgG purified by affinity column on such peptide coupled to EAH Sepharose gave precipitation line by Western blot analysis identical to that of N-myc oncoprotein, but it also reacted with c-myc oncoprotein. The implication of this experiment and prospect for further development of N-Myc ELISA will be discussed.

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GLUTATHIONE AND FERRITIN METABOLISM IN NEUROBLASTOMA TUMOR SAMPLES.

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Introduction
Ferritin and enzymes linked to glutathione metabolism: glutathione peroxidase, reductase, transferase and catalase and sodium dismutase were investigated in tumor samples obtained during surgery. The study was undertaken to examin the glutathione related drug resistance mechanisms and correlations with some neuroblastoma prognostic factors. Correlations of ferritin with glutathione enzymes and selected prognostic factors were also examined.

Material
Mentioned enzymes and ferritin were measured in 21 tumor samples of children representing different stages and histology. There were 5 stage II tumors, 6 stage III and 10 stage IV. The tumors were divided into Ganglioneuroblastoma (6) and Neuroblastoma (15) according to well known criteria.

Results
Results are sumarized in the table.

	AGE		P	HISTOLOGY		P	CHEMOTHERAPY		P
	<1yr	>1yr		GNB	NB		+	-	
GSTs total	n=4 201.0 +/- 26.0	n=17 346.0 +/- 47.0	S	n=7 369.0 +/- 109.0	n=14 297.0 +/- 33.0	NS	n=14 376.0 +/- 54.0	n=7 242.0 +/- 32.0	NS
GSTs basic	n=4 29.6 +/- 5.6	n=13 60.1 +/- 6.6	S	n=5 34.6 +/- 5.8	n=14 60.5 +/- 7.1	S	n=13 62.9 +/- 24.0	n=6 33.6 +/- 20.2	S
F	n=4 86.2 +/- 136.5	n=17 75.7 +/- 180.4	NS	n=7 11.1 +/- 14.8	n=14 114.0 +/- 195.9	S	n=14 84.7 +/- 197.9	n=7 78.9 +/- 111.2	NS
GSTs acidic	n=4 27.4 +/- 6.5	n=13 19.9 +/- 3.1	NS	n=5 24.0 +/- 3.0	n=14 20.5 +/- 3.0	NS	n=13 21.6 +/- 13.0	n=6 21.0 +/- 4.8	NS
GPX	n=4	n=17	NS	n=7	n=13	NS	n=13	n=8	NS
GR	n=4	n=17	NS	n=7	n=13	NS	n=13	n=8	NS
SOD	n=4	n=13	NS	n=7	n=12	NS	n=12	n=8	NS
CAT	n=4	n=16	NS	n=7	n=13	NS	n=13	n=8	NS

GPX=glutathione peroxidase, GR=glutathione reductase, GSTs=glutathione transferase, SOD=sodium dysmutase, CAT=catalase
F=ferritin, GNB=ganglioneuroblastoma, NB=neuroblastoma, (+)=with chemotherapy prior surgery, (-)=without chemotherapy, NS=statistically not significant, S=statistically significant

Conclusions:
1. The levels of GSTs basic form within tumor tissue correlates with the age less than 1 year and more differentiated histology.
2. The levels of GSTs basic form are higher after chemothrapy given, what may indicate the activity of2 this isoform in the drug resistnce mechanisms.
3. Ferritin levels correlastes only with histology.
4. No correlaions between glutatione enzymes and frritin were found.

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FLOW CYTOMETRIC ANALYSIS OF DNA CONTENT AND PROLIFERATIVE ACTIVITY IN CHILDREN WITH NEUROBLASTOMA

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Flow cytometric DNA analysis were performed in a retrospective study of formalin-fixed paraffin embedded archival tumor specimens of 45 patients with neuroblastoma diagnosed between 1978 and 1993 in order to evaluate the prognostic significance of DNA ploidy and cellular proliferative activity. DNA index ranged between 1 and 2.70. The DNA ploidy was aneuploid in 6 (13.3%) and diploid in 39(86.7%) samples. The percentage of the aneuploid tumors was significantly less than that was found in similar studies. The percentage of cells in the S phase (SPF) ranged between 1% and 78% with a median of 31%. The five year overall and event free survival rates for the whole group were %41 and %32.9 respectively. There was no significant difference between the five year overall and event free survival rates of patients with aneuploid and diploid neuroblastomas as well as between neuroblastomas with a high (SPF > %31) and low (SPF ≤ %31) proliferative activity. The results did not show the prognostic significance of neither DNA ploidy nor the cellular proliferative activity in neuroblastoma in contrast to other studies. Prospective and large studies are still needed to show the role of DNA ploidy on prognosis of childhood neuroblastoma.

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PCR BASED DETERMINATION METHOD OF N-*myc* COPY NUMBER IN NEUROBLASTOMA: ITS CLINICAL APPLICATION

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The study of N-*myc* gene amplification is a routine procedure in many established protocols for neuroblastoma patients before the commencement of any treatment. In order to obtain a method better than Southern hybridization, we developed a PCR based determination method of N-*myc* copy number, which is simple, requires only 500 ng of sample DNA and is feasible within 48 hours. Utilization of the newly developed competitive PCR procedure to study the N-*myc* gene to neuroblastoma patients was started since October 1994. Four children entered in this trial. To obtain the material, the choice of laparotomy vs. fine needle biopsy depended on the size, homogeneity and location of the tumor. Fine needle biopsy was tried wherever it is accessible and when we are confident enough to obtain genuine results. In a specific infant with a big adrenal neuroblastoma, cPCR was particularly useful as the tumor was diagnosed before mass screening where a possibility of unfavourable neuroblastoma is contemplated and the decision for treatment procedure was vital, and of course, it should be based on copy number of N-*myc*. cPCR method with a fine needle biopsy was carried out and a regular protocol of treatment could be started in this patient without delay after obtaining the result within 48 hours. In another patient with a growing hepatomegaly (stage IVS), cPCR was performed not with a needle biopsy but with laparotomy because of the heterogeneity of the metastatic hepatic parenchyma. We anticipate that cPCR is useful for the determination of N-*myc* copy number in neuroblastoma even with smaller amount of specimen obtained by fine needle biopsy and is superior to Southern hybridization.

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CD44 EXPRESSION IN NEUROBLASTOMA AND RELATED TUMORS

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Background: CD44 is a cellular adhesion molecule that exists in several isoforms and whose expression has been correlated with metastasis. Recent reports suggest a strong relationship between N-myc amplification in neuroblastoma(NB) and the expression of the CD44 molecule. We investigated the relationship between other known prognostic indicators of neuroblastoma(N-myc amplification, age, stage and survival) and CD44 expression in NB, ganglioneuroblastoma (GNB) and ganglioneuroma(GN).
Methods: Fresh non-fixed or formalin-fixed, paraffin-embedded specimens were studied in 36 patients with NB, GNB and GN. The specimens were stained with a monoclonal antibody recognizing the CD44 standard form (BU52, The Binding Site) or all of the CD44 isoforms(NCL-CD44-2, Novocastra Lab). The grade of positive staining in tumor cells was examined. This data was correlated with other prognostic indicators and mass screening data for neuroblastoma.
Results: Twenty six of thirty-four cases with NB and GNB showed strongly positive staining, eight NB cases had no CD44 staining. Two GN cases were positive. All 11 cases detected by mass screening showed strongly positive staining. There was a significant inverse relationship between the expression of CD44 and N-myc amplification (p=0.002). Staining related to survival age was more significant than for N-myc amplification. There was no statistical relationship between CD44 and the clinical stage.
Conclusion: Our data suggests that there may be a relationship between CD44 expression and N-myc amplification. Moreover, it suggests that there is a relationship to age, survival and mass screening, too. However, there does not appear to be a relationship between CD44 expression and the clinical stage.

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DEVELOPMENT OF TUMOR VACCINATION STRATEGIES BY EX VIVO GENE TRANSFER OF IL-2 AND IFNγ INTO PRIMARY NEUROBLASTOMA CELLS

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Many aspects of neuroblastoma behavior such as spontaneous regression of low-risk tumors or susceptibility to cytotoxic effector mechanisms support an immunologic approach to neuroblastoma treatment. Animal models suggest that tumor growth can be inhibited by the expression from neuroblastoma cells of IL-2 which is costimulatory for cytolytic effector lymphocytes, or IFNγ which causes tumor rejection by reversal of N-myc mediated down regulation of MHC I expression.

In our experiments tumor cells are transduced ex vivo by use of transferrin-dependent receptor-mediated endocytosis. This is the process by which the human iron-transport protein transferrin is covalently linked to the DNA-binding molecule polylysin and DNA-polylysin-transferrin complexes are transferred actively into cells. Coupling of a replication-deficient adenovirus to the DNA-polylysin-transferrin complexes results in disruption of endosomes upon endocytosis and admits DNA into the cytoplasm and eventually into the nucleus yielding up to 100% transfection of various cell lines.

We were able to transduce human and murine neuroblastoma cell lines as well as primary human neuroblastoma cells collected from patients. The reporter genes luciferase and β-galactosidase and the cytokines IL-2 and IFNγ could be detected in considerable amounts. Exposure to IFNγ, furthermore, caused increased expression of MHC I molecules from primary human neuroblastoma cells. We next will test these tumor vaccines in a neuroblastoma mouse model to further explore possibilities for clinical applications of the concept of tumor vaccination.

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ALLOGENEIC BONE MARROW TRANSPLANTATION FOR ADVANCED NEUROBLASTOMA

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Eleven Japanese centers use high-dose chemoradiotherapy with allogeneic bone marrow transplantation (BMT) for advanced neuroblastoma. The aim of this study was to determine (1) the sites and frequency of tumor recurrence after BMT, and (2) toxicity and efficacy of BMT. Patients and methods: From April 1986 to February 1995, 30 patients (age > 1 year) with stage IV neuroblastoma received BMT; 19 patients were in 1st complete remission (CR), ? were in 2nd CR, and 8 had residual disease. Twenty-nine underwent transplantation from HLA-identical siblings. The preconditioning regimen consisted of melphalan (± other agents) and total body irradiation (TBI) at median doses of 180 mg/m^2 and 12 Gy, respectively. As prophylaxis against graft-versus-host disease (GVHD), patients received methotrexate (MTX, n=25) or MTX + cyclosporin A (n=5). Results: Three-year disease-free survival after BMT was 46.4%. Twelve children relapsed, and 15 sustained remission for 11 - 120 months. Relapse rate did not differ in CR (9/22) vs. non-CR (3/8) cases. The addition of other agents to melphalan and TBI showed no benefit. In 27 cases evaluated for TBI, the rate of relapse was higher after doses of < 12 Gy (5/6) than ≧12 Gy (7/21); (p=0.06). Relapses to bone, bone marrow, and other sites occured in 9, 8, and 4 cases, respectively. The 1st site of relapse was variable: previously affected bone (n=3), new bone sites (n=5), bone marrow (n=5), and other (n=1); two cases metastasized to two sites simultaneously. Acute GVHD (≧II°) and chronic GVHD occured in 8 and 2 cases, respectively. Severe stomatitis or mucositis related to treatment developed in 5 patients, and renal dysfunction in 6. 15 patients died of disease: relapse in 9 cases, relapse plus other causes in 3, and regimen-related toxicity (severe infection) in 3.

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THE EVALUATION OF COMPREHENSIVE TREATMENT FOR STAGE 1V NEUROBLASTOMA

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The study was based on the analysis of our remarkably improved survival rates for stage 1V neuroblastoma (NB). We experienced 28 cases with stage 1V NB from 1965. Since 1987, we introduced supralethal chemotherapy with BMT or PBSCT. From the results of the therapy, 6 cases out of 8 with stage 1V survived and achieved long-term survival of 8 years, 7, 6, 3 , 2.2 years respectively. The scheme of our therapy: the tumor tissue was taken firstly by open biopsy and examined single cell suspension assay (MTT-assay) to evaluate the appropriate chemotherapy. According to MTT-assay, the patients were treated with regimen A1 (consist of CPM,VCR,ADM,CDDP) or regimen new A1 (consist of CPM,VP16,ADM,CDDP). Surgical resection of the primary tumor was performed when the resolution of distant metastasis has been obtained. Supralethal chemotherapy consisting of L-PAM, VP16 selected by MTT-assay was designed with BMT or PBSCT to achieve final goal. Two cases out of three with N-myc amplification over 64 copy and 5 cases with bone metastasis survived. We concluded that appropriate, supralethal chemotherapy combined surgery was available to improve the prognosis even for the unfavourable advanced NB.

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SOMATOSTATIN RECEPTOR SCINTIGRAPHY (SRS) IN NEUROBLASTOMA (NB): PRELIMINARY RESULTS

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High affinity somatostatin receptors (SR) have been characterized on neuroendocrine cells and related tumors (e.g. NB). Long acting somatostatin analogues labelled with radionuclides are in use as diagnostic agents in SR expressing tumors in adults. We report our results of SRS in 39 patients with NB (6 St. 1, 7 St. 2, 6 St. 3, 18 St 4, 2 St. 4S according to INSS) at diagnosis (25), PR (2), CR (5), relapse (5) and with progressive disease (2). For SRS, an ^{111}In labelled somatostatin analogue has been used (^{111}In-Pentetreotide) with an average dose of 30 MBq. Scans have been performed 2 -20 hours post injection. All patients underwent ^{123}I-MIBG-scintigraphy as well within 1 week. MYCN and/or DNA ploidy and/or integrity of chromosome 1p, parameters of prognostic relevance were investigated in all tumors. 8 pts were both negative in MIBG and SRS respectively. All others (31) were positive for MIBG. 21/31 showed high and 3/30 low SR expression. 7/30 MIBG positive tumors showed no SR expression. No MIBG negative/SRS positive NB was detected. Interestingly all MIBG positive NB with no or low SR expression revealed at least one prognostic unfavourable biological feature. Our data suggest that no or low expression of SR as demonstrated scintigraphically in MIBG positive NB may be correlated with more unfavourable outcome in NB. The discordant results in MIBG vs. SRS and the possible correlation of SRS with prognosis suggests that SRS could provide additional information as an in vivo biological prognostic parameter in neuroblastoma. This would be of interest when deciding about treatment for children with localized NB especially those detected by mass screening.

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HOW SHOULD WE TREAT NEUROBLASTOMA DETECTED BY MASS SCREENING?

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OBJECT: To answer the question about overtreatments or unnecessary treatments for neuroblastoma detected by mass screening (MS), we have decided to observe them without operation and chemotherapy when they satisfy our criteria.

PATIENTS AND METHODS: We experienced 25 neuroblastoma cases detected by MS at 6 months of age from 1991 to 1995. All 25 cases are alive. At the beginning all patients were operated with or without chemotherapy. However, since 1994, 4 cases who satisfied our criteria; 1)the diameter of the primary tumor is less than 3cm, 2)no apparent vascular or organic invasions, 3) informed consent is acquired, were selected to observe.

RESULTS: Among observed 4 cases, 2 cases including stage4S and stage1 were operated 199 days and 291 days after the 1st screening respectively, because of enlargement of tumor and elevation of tumor markers. The remaining 2 cases including stage1 and stage2 are still under observation for 290 days and 517 days respectively. Tumor sizes are reducing and tumor markers are decreasing gradually.

CONCLUSION: Some cases detected by MS may regress spontaneously. With careful observations we are currently extending the present study.

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SCREENING FOR NEUROBLASTOMA INCREASES INCIDENCE OF THE DISEASE UNDER 1 YEAR INFANTS WITHOUT REDUCING FOR 1 TO 4-YEAR-OLD CHILDREN

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Neuroblastoma mass screening for 6-month-old infants was initiated in Saitama Prefecture, Japan, in June 1981. Urinary VMA was assessed qualitatively until September 1989 and after that VMA/HVA were measured qualitatively. To evaluate effect of the screening we compared the incidence rates(/10^5) for children under 5 years of age born from 1981 to 1985(cohort A) with that born from 1986 to 1990(cohort B). Approximately 365 thousands infants were screened with 36.7 and 77.4 % compliance in A and B cohort respectively. Fifty three and 75 cases were detected in A and B cohort respectively. In A cohort, 29 cases(14 screening and 15 clinical) were under 1 year, and 24 cases(one screening and 23 clinical) were 1 to 4 years of age. In B cohort, 51 cases(40 screening and 11 clinical) were under 1 year and 24 clinical cases were 1 to 4 years of age. Statistical significant increase were observed in the incidence for 0 to 4 years of age (A:14.5, B:23.0, P=0.018), and in infants under 1year(A:8.2, B:16.0, P=0.005). No reduction was observed in incidence for 1 to 4 years of age (A:6.3, B:7.0, P=0.872) and in that of clinical cases for under 1 year. In conclusion, neuroblastoma screening increased incidence of the disease by detecting a group of tumors which would regress or mature and could not be diagnosed later clinically.

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NEUROBLASTOMA SCREENING AT ONE YEAR OF AGE IN GERMANY: STUDY DESIGN AND PRELIMINARY RESULTS.

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Mass screening of infants for early detection of neuroblastoma has been started in Japan in the early 1970s and more than 1 million children are tested every year by this procedure in Japan. Studies were initiated over the last years in Canada, U.K., France, Austria, USA, Australia, Italy, Norway and Germany. The results of these studies show, that neuroblastoma can be diagnosed early by detecting urinary catecholamine metabolites. The Japanese and Canadian data indicate the possibility of overdiagnosis of subclinical, possibly spontanous regressing neuroblastomas if the screening is performed before 6 months of age. For several reasons (e.g. incomplete cancer registration) no statement about a decrease in mortality could be made so far. The newly initiated German project is an epidemiological study to evaluate the presumed benefit from of neuroblastoma mass screening at 1 year of age. 1.25 million screened individuals (by HPLC) in the study regions will be compared to the same number of not screened children in the control regions within 4 years. The German Children's Cancer Registry enables a mostly complete follow up of all neuroblastoma patients therefore conclusive results of the impact on mortality can be expected. Rules have been introduced to define overdiagnosis. Within the first 9 months 168,358 children were tested and 12 neuroblastomas could be detected by screening. The results of this screening program will be crucial for the implementation of neuroblastoma mass screening in the general preventional program for children in Germany. (Supported by Deutsche Krebshilfe)

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PLASMA CHROMOGRANIN A LEVELS ARE DIRECTLY PROPORTIONAL TO TUMOR BURDEN IN NEUROBLASTOMA

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In neuroblastoma, biochemical markers are used for diagnosis and monitoring. The aim of this study is to investigate the usefulness of six neuroblastoma markers in a new animal experimental model. The markers investigated were chromogranin A (CgA), a protein co-stored and co-released with catecholamines, pancreastatin (Ps), neuron-specific enolase (NSE), homovanillic acid (HVA), vanillyl-mandelic acid (VMA), and dopamine (DA). Thirteen nude rats (WAG rnu/rnu) were injected s.c. with human neuroblastoma cells (SH-SY5Y), and seven animals served as controls. Tumor volume was measured with caliper every other day. Animals were put in metabolic cages for 24-hr urine collection every fourth day. A blood sample was drawn before returing the animal. CgA and Ps were measured by specific RIAs developed by M.S., NSE with a commercial ELISA kit. HVA, VMA, and DA were measured by HPLC. Tumor take was 88% (23/26 injections). CgA was directly proportional to tumor volume ($r=0.83$, $p<0.001$). CgA rose in parallel with tumor growth. Ps was not detectable in any animal. NSE was elevated in tumor-bearing animals ($p<0.01$) but did not correlate with tumor volume ($r=0.49$, $p>0.05$). HVA was elevated in tumor-bearing animals ($p<0.01$), but did not correlate with tumor volume ($r=0.32$, $p>0.05$). VMA was not detectable. DA was found in low concentrations that did not correlate to tumor volume. CgA is a new and promising neuroblastoma marker since its concentrations were directly proportional to tumor burden and rose in parallel with tumor growth. A clinical investigation of the use of CgA in neuroblastoma has been initiated.

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CT AND MIBG-SPECT IMAGE FUSION IN NEUROBLASTOMA : WORK IN PROGRESS

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PURPOSE :
1. To combine the functional (MIBG-SPECT) with the morphological (CT) informations in Neuroblastoma.
2. To study the feasibility in children with altered clinical status.
3. To test the diagnosis efficacy of the fusionated data.

MATERIALS & METHODS : 12 patients with neuroblastoma of the trunc were studied from Sept.1st 1995 to Feb.19th 1996. The injected radioactivity was 0.1 mci/kg for I^{131} and 0.25 mci/kg for I^{123}. SPECT tomography was focused on abnormal contrast uptakes. Volumic CT aquisitions used 5mm slices with extended helix, and overlapped reconstructions. Four multimodality radiographic markers filled with I^{131} were sticked on the skin. CT and SPECT-tomography were performed on separate days. Immobility was obtained with usual I.V. sedation. No breath holding was required.

RESULTS : Image quality was excellent in every case without motion artifact on both CT or SPECT-tomography. Concordance of each external marker was reached in every case (discrepancies inferior to 5 mm).
Centers of MIBG uptakes correlated well with centers of morphological lesions whenever visible. Margins of both morphological lesions and MIBG uptakes didn't correlate as far as the diameters of contrast uptakes correlates only with intensity of radionuclide tumoral captation. The most functional parts of the tumors could be located before diagnosis biopsy in 3 cases. Dubitous topography of SPECT uptakes facing adjacent organs could be precisely located with fusion in 5 cases.

CONCLUSION : These preliminary results show that technical management is feaseable in sick children and improvement of clinical informations was provided in most cases.

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NEUROBLASTOMA SCREENING AFTER THE SIXTH MONTH OF AGE DETECTS "UNFAVORABLE " NEUROBLASTOMA CASES.

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The Japanese neuroblastoma screening program which is performed at the infants' age of six months has led to early detection of more than one thousand neuroblastoma cases. However, most of these tumors were "favorable" neuroblastomas. An increase of neuroblastoma incidence has led to the suspicion that early neuroblastoma screening might predominantly detect neuroblastomas which otherwise would regress spontaneously. As a consequence the Austrian screening program was postponed after the sixth month of age. Between January 1991 and January 1996 screening was performed in 127,941 infants aged between seven and twelve months. For analysis of vanillylmandelic acid (VMA) and homovanillic acid (HVA) we used EIA, HPLC and GC-MS method. Cut-off values were determined for every method by use of mean value + 2.5 SD. Due to repeatedly elevated urine catecholamines 32 infants were admitted to a hospital for clinical investigations. In 17 cases a neuroblastoma was found. All tumors were abdominal tumors, 8/17 of adrenal origin, 9/17 of extraadrenal origin. According to the INSS four cases were stage 1, five stage 2B, seven stage 3, and one stage 4. According to the Shimada and Joshi classifications 2/12 tumors showed unfavorable histology. Analysis of biological features revealed N-*myc* amplification in 3/16 cases and near-tetraploidy in another case. When stage >=3, elevated LDH, HVA/VMA ratio > 1, unfavorable histology, N-*myc* amplification, 1p deletion and di-/tetraploidy were considered as independent risk factors. 13/17 patients had at least one risk factor. 8/17 infants received chemotherapy due to tumor unresectability or gross residual tumor. One patient died as a consequence of excessive intraoperative bleeding, the other 16 patients are alive with a median follow-up of 16 months. However, one former stage 2B patient presented with multiple metastases seven months after macroscopically complete resection of the (near-tetraploid) primary tumor. One false-negative case was observed so far (negative screening result at the age of 12 months, but clinically presenting stage 4 neuroblastoma 27 months later).
Our results demonstrate that neuroblastoma screening after the sixth month of age is able to detect more cases with unfavorable biological features in which spontaneous regression is unlikely.

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RECOMBINANT HUMAN ALPHA INTERFERON (rHuαINF) IN CHILDREN WITH ADVANCED NEUROBLASTOMA FOLLOWING INTENSIVE THERAPY AND AUTOLOGOUS STEM CELL REINFUSION (ASCR)

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Introduction: High-dose chemotherapy followed by ASCR after conventional treatment has improved the prognosis in stage IV neuroblastoma (NBL) patients (pts). However, non eradicated minimal residual disease (MRD) will be responsible of late relapse in 45-60% of the cases.
αINF is known to stimulate natural killer cells (NK) in their cytolytic antitumoral activity. Following high-dose chemotherapy with ASCR, a preferential outgrowth of NK cells with both NK and lymphokine-activated killer cells activity is observed. αINF given after autograft could thus represent a new therapeutic strategy for MRD. We report the preliminary results of our phase II clinical trial which tested the toxicity and potential role of rHuαINF in eradiacating MRD.
Methods, patients: 9 children with stage IV NBL in 1st CR (4 pts), 2nd CR (1 pt) or VGPR (2 pts) and stage III NBL in 2nd CR (2 pts) were included into the study after ASCR, when the thrombocyte count reached $\geq$ 50 G/l and absolute neutrophile count $\geq$ 1 G/l. They received subcutaneous injections of rHuαINF 3x/week at a dose of 3 Mio U/m^2 for 1 year. Hematologic, hemostatic, hepatic and renal parameters were controlled 1-2x/month, the diesease status every 3 months.
Results: The median time interval between the end of tratment (=ASCR) and study entry was 7.5 months (range 3 weeks - 26 months). Only 1 local transitory reaction after injection was observed. Three out of 9 pts had to be taken off study for either relapse (1 pt) or grade IV toxicity (2 pts). Among the remaining pts, 3 are in CR and 1 in VGPR 11.5 months (range 7-17 months) after the end of rHuαINF, 2 are still on treatment. Among the toxic reactions, 6/9 pts presented a grade 1 (1 pt), 2 (2 pts), 3 (1 pt) and 4 (2 pts) transitory thrombocytopenia resulting in interruption of the study only in 1, and 2/9 a grade 2-3 transitory hepatotoxicity. An unexpected transverse myelitis was observed in one child receiving concommitant local radiotherapy requiring an immediate interruption of the treatment and amendment of the protocole for radiotherapy.
Conclusion: Our preliminary results show that rHuαINF is well tolerated and that its main toxic effect is transitory thrombocytopenia. The low incidence of relapse (1/7 pts) justifies the continuation of the study, whereas it is too early to evaluate the efficacy of rHuαINF in terms of better survival of the children.

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ADOPTIVE IMMUNOTHERAPY WITH RECOMBINANT HUMAN IL-2 (rHuIL-2) AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR HIGH-RISK NEUROBLASTOMA (HR-NB).

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Objective. A phase II/III trial, with low doses of rHuIL-2 over a prolonged period of time, was carried out. The aim of the study was to evaluate immunomodulatory effects induced by IL-2 and its potential role in eradication or control minimal residual disease (MRD) in NB after high dose chemotherapy (HDCT) followed by ASCT.

Patient and method. From 1/92 to 2/96, 25 pts with HR-NB (1 in III CR, 2 in II VGPR, 2 in II CR, 13 in I VGPR, 7 in I RC) received rHIL-2 (Proleukin, Aldesleuchina), after a median (min-max) time of 91 (43-153) days from ASCT. Treatment schedule consisted of 2 cycles of 24-h iv for 5 days (2-4-6-8-8 MU/sqm/d respectively) followed by 11 monthly and 6 bimonthly cycles of rHuIL-2 administered sc for 5 d (2-4-4-4 MU/sqm/d), for a total of 18 cycles/pts.

Results. Were administered 223 cicles of therapy. Immunological analysis globally evidenced an increment of NK and activated T cells number. Iperpirexia (25/25) and trombocytopenia (4/25), were the only rHuIL-2 dependent toxicity observed during iv or sc administration. 1 pt stopped the iv phase for gram- sepsis, 1 pt reduced iv dosage because a feverish convulsion. 20/25 pts are alive and well with a median (min-max) follow up of 10 (2-50) months, 5/25 pts relapsed with a median (min-max) time of 13 (6-41) months, only one of these pts died for PD, 35 months after ASCT. Overall EFS at 3 years was 33%.

Conclusion. Adoptive immunotherapy with low doses of rHIL-2 is feseable and seems to be effective in inducing activated immunocompetent cells proliferation and in controlling MRD after HDCT and ABMT in HR-NB.

(Supp. by Associazione Italiana Neuroblastoma)

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I^{131} MIBG TREATMENT IN 8 CHILDREN WITH ADVANCED NEUROBLASTOMA

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INTRODUCTION: A response to I^{131} MIBG therapy has been reported in patients with resistant neuroblastoma. OBJECTIVE: To measure the therapeutic response to I^{131} MIBG in children with progressive stage III or IV neuroblastoma. PATIENTS AND METHODS: Seven boys and one girl aged 17 to 92 (mean 39) months at diagnosis with stage III (n2) or IV (n6) disease were given one to 13 courses of I^{131} MIBG (total dose 2775-54151 mBq) after failed chemotherapy (n7) or immediately after surgery (n1). Urinary VMA/HVA was elevated in 5 stage IV and one stage III patient. Marrow infiltration was present in all stage IV patients. Increased I^{123} MIBG uptake was present in the abdomen of all, ± other areas eg marrow, skeletal sites, thorax. The interval between diagnosis and I^{131} MIBG therapy was 1 week to 3½ yr. Lugol's solution was used to protect the thyroid. RESULTS: The dose of I^{131} MIBG and the improvement (+) or not (-) in 5 measurable parameters are listed in the table. Five children had marked improvement in 3 or more parameters. Patient no 4 needed a red cell and platelet transfusion and patient no 3 had transient thrombocytopaenia (50-100X10^9/ℓ). Hypothyroidism developed in patients 1, 3 and 5. Six patients have died of disease 3 to 21 (mean 10) months after onset of MIBG treatment. Two patients are alive with residual disease.

No	I^{131} MIBG mBq	PAIN	TUMOUR SIZE	MARROW INFIL-TRATION	HVA/MVA URINE	I^{123} MIBG UPTAKE
1	6880	+	+	+	+	*NT
2	2775	+	+	_	_	+
3	54151	+	+	+	_	+
4	6665	+	+	_	_	+
5	13303	_	_	*NP	*NR	_
6	10167	+	+	+	+	+
7	8119	_	_	*NP	*NT	_
8	2775	*NP	_	+	+	_

***NT = Not tested *NP = Not present *NR = Not raised. CONCLUSION: Objective responses were obtained with little toxicity and morbidity. I^{131} MIBG therapy in advanced neuroblastoma needs further investigation.**

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THERAPY FOR NEUROBLASTOMA INFANTS IN JAPAN CURRENT STRATEGY ON THE JAPANESE INFANTILE NEUROBLASTOMA COOPERATIVE STUDY (JINCS)

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Neuroblastoma(NB) infants in Japan had been treated relatively intensive. To standardize and to optimize therapy for infantile NB in Japan, the nationwide prospective study (#9405) was opened in June 1994. Chemotherapy is stratified according to N-*myc* amplification (≥10 copies) and clinical stage. 1) For patients without N-*myc* amplification: After a complete removal of tumor, stage 1 patients receive no adjuvant chemotherapy, while stage 2 patients are randomized into two groups followed with or without a minimized adjuvant chemotherapy of Regimen A (VCR/CPM) for 3 months. Stage 3 patients with a resectable tumor are randomized into two groups receiving either Regimen A or B(CPM/ADR) for 6 months. Stage 3 patients with an unresectable tumor are preoperatively treated with Regimen C (VCR/CPM/ADR), and then randomized as the same as for patients with a resectable tumor after surgery. Stage 4 patients with or without bone metastasis receive Regimen C or D (VCR/CPM/ADR/ CDDP). Stage 4s patients with a resectable primary tumor receive surgery followed with Regimen A for 3 months. Stage 4s patients with an unresectable tumor receive preoperatively Regimen C and then postoperatively Regimen A as the same as for patients with a resectable tumor. 2) For patients with N-*myc* amplification: All patients in any stage receive Regimen D. By the end of January 1996, 94 cases were enrolled. There were 79 cases found through mass screening, while 15 cases found clinically. Seventy-nine patients were in localized stages 1, 2 and 3, while 6 and 9 in metastatic stages 4 and 4s. N-*myc* amplification (≥10 copies) was found in only one out of 85 cases tested. DNA polidy was assessed in 75 cases, and diploidy pattern was observed in 12 cases. Chromosomal analysis was successful in 49 cases and revealed abnormal karyotypes in 21 cases, including 1p deletion in 5 cases. Histopathological classification of Shimada in 56 cases found only one case with unfavorable histology. There is, so far, no fatal and relapsed cases reported. This prospective study would be expected to standardize as well as to optimize therapy for infantile NB in Japan.

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DISSEMINATED POOR-RISK NEUROBLASTOMA WITH UNDETECTED PRIMARY. A "SPECIAL" PATIENT SUBSET?

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Background. Unlikely from adults, metastatic cancer with undetectable primary is quite uncommon in children. Neuroblastoma is the pediatric tumor where this seems to occur more frequently, but little is known about the clinical and biological characteristics of children with this type of presentation.

Methods. The Italian Neuroblastoma Registry (21 Italian institutions) was scrutinised to detect children aged 1-15 years with newly diagnosed disseminated disease but no primary. Infants and stage 4s patients were excluded from this analysis.

Results. Of 470 such children diagnosed between 1979-1995 eight (0.17%) had no detectable primary. There were 7 boys and one girl. Age ranged between 21-90 months (median, 33). Bone marrow was the only site of the disease in one case, both bone and bone marrow were involved in the other 7 including one having also orbital involvement and another having liver and superficial nodes involvemen. All had elevated urinary VMA excretion. MYCN gene was assayed in only one child and was amplified. All patients either responded poorly or did not respond at all to chemotherapy. Only one is alive with disease at 12 months. The remaining 7 died at 5-19 months from diagnosis (median, 12). In no case a "primary" tumor developed during the disease course.

Conclusions. Very few children with disseminated neuroblastoma have no detactable primary. These tumors display poor chemosensitivity and great aggressiveness and may represent a distinct patient subset. The identification of specific characteristic encounters practical difficulties that could possibly be overcome by new technologies enabling the study of individual tumor cells in metastatic sites.

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Histological prognostic factors of neuroblastoma

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Recent clinical investigations suggest that most neuroblastomas found in infants at 6 months of age by VMA/HVA mass screening (ms) show extremely benign clinical behavior. In this study, focusing upon the differentiation/maturation process and apoptosis, we analyzed the histological features of neuroblastoma in patients examined between 1985 and 1994. We examined 63 ms patients (7.1 ± 1.3 mo), and 35 non-ms patients (3.3 ± 3.3 yr). There were no tumor deaths among the ms patients, compared with 16 deaths among the non-ms patients. We used four antibodies for immunohistochemical studies. The in situ end-labelling (ISEL) method was used for demonstration of apoptosis.

The rates of expression of S-100 protein and microtubule-associated protein-2 (MAP-2) were significantly high in ms cases. There was no significant difference in the expression of vasoactive intestinal polypeptide (VIP) and neural cell adhesion molecule (NCAM) or in that of apoptosis between ms and non-ms patients. Among non-ms cases, patients whose tumor showed no expression of S-100, NCAM, and MAP-2 had an poor prognosis. Among the three antigens examined, lack of MAP-2 expression was indicative of a very poor prognosis.

It was suggested that tumor differentiation/maturation was progressive in ms cases, and that MAP-2 expression was the most striking factor correlated with patient prognosis.

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TURKISH EXPERIENCE WITH NEUROBLASTOMA: AN ANALYSIS OF 125 PATIENTS FROM A SINGLE INSTITUTION

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Clinical characteristics and treatment results of 125 patients with neuroblastoma diagnosed and treated between 1977 to 1996 were evaluated. Median age of the patients was 2.5 years (range 1.5 months-13 years). Male to female ratio was 1 (73/72). Ninety-six (76.8%) of tumors were abdominal, 20 (16%) thoracic, 5 (4%) cervical, 3 (2.4%) pelvic. Histopathological investigation revealed the diagnosis of neuroblastoma in 109 (87.2%) of patients. Fifteen (12%) patients had ganglioneuroblastoma and 1 (0.8%) patient ganglioneuroma. Five (4%) patients were stage I, 28 (22.4%) stage II, 20 (16%) stage III, 52 (41.6%) stage IV and 20 (16%) stage IV-S. Seventy-two patients (57.6%) had distant metastasis at the time of diagnosis. The most common sites of metastasis were bone (16.8%), liver (19%), bone marrow (12%), and soft tissue (12.8%) followed by orbit (8%), lymph nodes (5.6%), meninges (2.4%) and testis(1.6%). Neuron specific enolase was >100ng/ml in 13 of 42 (30.9%), ferritin was >150mg/dl in 16 of 37 (43%), urine VMA spot test was positive in 51 of 77 (66.2%) and quantitative urinary VMA was positive in 30 of 54 (55.5%) of the patients. In 40 cases flow cytometric DNA Ploidy analysis was performed and aneuploidy was found in 29 (72%) patients. Aneuploidy correlated with younger age and early stages. Surgery was performed initially in 93(74.4%) patients, in 53 of whom biopsy was the only performed procedure. Twenty-seven patients were treated with CYC+VCR, 29 with COMP, 32 with CYC+VCR+DDP and 5 with CYC+VCR+DTIC+ADR+VM26 at conventional doses until 1990. From 1990 to 1996, 21 patients were treated according to the intensive German Cooperative Study NB90 protocol. Nine patients died before initiation of chemotherapy. The long term overall survival of all patients was most closely related to stage, being 100% for stage I, 82 %for stage II, 15% for stage III and 14% for stage IV. The probability of survival in patients with advanced disease, however, was significantly different between the patients treated with the conventional protocols until 1990 and NB90, 10.5% vs 33% for Stage III and 1% vs 35% for Stage IV respectively. Hyperdiploidy also showed correlation with fatal outcome. It has been possible to improve prognosis in advanced stage neuroblastoma with intensively dosed chemotherapy an association with surgery and radiotherapy plus good supportive care.

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FINE NEEDLE ASPIRATION CYTOLOGY IN THE DIAGNOSIS OF ABDOMINAL AND RETROPERITONEAL MASSES IN CHILDREN

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We reviewed 80 cases of FNAC of abdominal and retroperitoneal masses, selected from a series of 622 FNAC performed in our pediatric oncology clinic between January 1990 and August 1995. There were 38 boys and 42 girls, aged 3 months to 16 years. FNAC was used for the initial diagnosis in all cases. Ancillary studies, including immunocytochemistry (n=39), electron microscopy (n=22), cytogenetics (n=3) and microbiologic culture (n=1) were done on the aspirated material. Of the 80 aspirates, 2 were inadequate, 5 were diagnosed as benign lesions, 71 as malignant tumors and 2 were inconclusive. In 74 out of the 78 adequate aspirates, a definitive diagnosis was done, either by cytomorphology alone, or supported by complemmentary techniques. The following primary diagnosis were made: lymphoma (n=25), neuroblastoma (n=16), Wilms' tumor (n=12), hepatoblastoma (n=4), germ cell tumor (n=6), rhabdomyosarcoma (n=4), PNET (n=2), Hodgkin disease (n=1), TCRP (n=1), fusiform sarcoma (n=1) and benign lesions (n=3). The 2 inconclusive cases proved to be an hemangioendothelioma and a gonadoblastoma. These results yield a sensitivity of 97,2 %, a specificity of 100 % and an accuracy of 97,4 %. We think FNAC is a valuable method in the diagnosis of abdominal masses in children because is a minimally invasive procedure and achieves an accuracy identical to the published results obtained by biopsy.

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FINE NEEDLE ASPIRATION (FNAC) IN PEDIATRIC ONCOLOGY. REVIEW OF 6 YEARS

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The use of FNAC in the diagnosis of tumoral masses has recently been extended to the pediatric group. From January 1990 to November 1995, FNAC was performed on 707 children (age, 2 month-16 years; mean: 9,4)refered to our pediatric oncology unit on the suspicion of malignant lesion. The aspirated sites were: lymph node (n=193), bone and soft tissue (n=158), thyroid (n=101), testis (n=77), abdominal cavity (n=79), head and neck (n=53), breast (n=30) and thorax (n=16). In 530 of the 707 cases FNAC was used for the initial diagnosis.
Ancillary studies, including immunocytochemistry (93), electron microscopy (50), microbiologic culture (10) and cytogenetic's (7) were done on the aspirated material. Five hundred eighty seven (95%) aspirates were adequate for evaluation. 354 corresponded to non-tumoral lesions and 306 to neoplastic diseases, being 267 malignant and 39 benign. In the remaining 14 cases a conclusive diagnosis was not achieved. The sensitivity of pediatric FNA was 96%, specificity, 100% and accuracy of the method, 98%. This series supports the premise that cytopathology in conjuction with ancillary studies (EM, ICC), is a valid technique for the diagnosis of childhood tumors, when it is applied by experienced practioners with a good clinico-pathological dialogue.

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COMBINED TREATMENT: CHEMOTHERAPY + RADIOTHERAPY + HDR BRACHYTHERAPY ON CHILDHOOD NASOPHARYNGEAL CARCINOMA

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Purpose: Nasopharyngeal carcinoma is an uncommon disease on childhood. Radiotherapy is the treatment of choice but the survival and local control rates are disappointed. This report presents the results of a combined treatment approach for nasopharyngeal carcinoma in children using chemotherapy followed by external beam radiotherapy and HDR brachytherapy.
Material and Methods: Seven children with primary nasopharyngeal carcinomas treated from september/92 to september/95 are reported. The median age was 14 years with 3 male and 4 female. The treatment included chemotherapy with Mitoxan/Vincristin/Adriamycin/CDDP each 21 days. After three cicles the patients received external beam radiotherapy to the primary site and cervical region. The dose to the nasopharinx and to the neck was 45Gy to 50Gy and metastatic neck nodes received adtional 10Gy. HDR brachytherapy was performed two weeks after external radiotherapy using a remote controled Ir192 source. A metalic aplicator was introduced by nasal via under local anesthesia. The correct position of the catheters and the definition of interest points were confirmed by orthogonal X-rays. A median dose of 6Gy (5Gy - 7,5Gy) was prescribed to 5mm from the catheters. Five patients received two insertions with a two weeks interval and two patients received only one insertion. After brachytherapy all children received three cicles more of chemotherapy.
Results: The treatment was well tolerated. Cutaneous descamation and hyperpigmentation of the radiotherapy fields associated with reduction of salivary production were observed. With a median follow-up of 15 months (3 - 27 months) 7/7 patients (100%) show local control of the disease and 6/7 patients are alive with no tumour. Only one patient is dead by cardiotoxity of chemotherapy.
Conclusions: Combined treatment of nasopharyngeal carcinoma is well tolerated with low incidence of side effects. HDR brachytherapy possibilities to reduce the dose of external radiation to the nasopharinx increasing the local control. Longer follow-up is necessary to definitive conclusion but HDR brachytherapy needs to be estimulated on treatment of nasopharyngeal carcinoma on childhood.

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TREATMENT MEHTODS IN BILATERAL RETINOBLASTOMA: RESULTS OF COMBINED TREATMENT OF 77 CHILDREN.

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The problem arises due to difficulty in prognosis the effectiveness of combined treatment and danger of surgical removal of the most radiosensitive tumor in one of the eyes in children with bilateral retinoblastoma (RB). 77 patients have T3 primary tumor stage in one or both eyes. 75,3% children were undergone conservative (chemotherapy and radiotherapy) treatment (CRT). The others (24,7%) - surgical removal of the most affected eye with secondary complications (uveitis, glaucoma, hemophtalm). For radiotherapy Cobalt-60 or 13 mv-electrons were used. Daily dose was 1,5-2,0 Gy, total dose was 40-50 Gy. Simultaneosly conservative therapy includes cyclophocphamide, vincristin and adriamycin for primary tumors, medicaments of platinum and metatrexat for relapse. Medicaments were used intravenously in standart dose 6-8 cycles during 24 months. Complite and partial response without any signs of progressing disease during 2 and more years was observed in 67,5% of patients. 32,5% patients had a local relapse in one of the affected eye after 12-18 months of treatment. In these cases enucleation was performed. No cases of bilateral relapse were observed. In some cases the best therapeutic effect was found in one of the eye which was more affected. The attempt of conservative treatment of bilateral RB in noncomplicated cases on the first stage does not aggravate the prognosis of the desease on the whole preserving visual organ. 5-years survival of children with retinoblastoma in CRT was 83% and after enucleation on the first stage of treatment was 84%.

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RETINOBLASTOMA IN AFRICAN CHILDREN : PRELIMINARY RESULTS OF MOLECULAR ANALYSES

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Retinoblastoma comprises 3% of all childhood cancers (under 15 years) in caucasians according to the SEER data. At Kalafong Hospital, Pretoria, retinoblastoma was recorded in 17% of all cancers in African children under 12 years of age, seen over a 10 year period. A study is presently being undertaken to ascertain the causative mutations in the Rb-gene in African retinoblastoma patients. Using exon-by-exon PCR-SSCP analysis three new mutations have been detected to date. These comprise one somatic and two germline mutations. The two germline mutations were detected in bilateral stage IV retinoblastoma patients, aged 19 months and 4 years, respectively, and the somatic mutation was in a 18 month old child with unilateral stage II disease. These patients do not have any significant family history of retinoblastoma. These preliminary results, of previously unreported mutations in the retinoblastoma gene, preface the need for further research to illustrate the underlying molecular basis of retinoblastoma in the African child.

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THE OUTCOME OF PATIENTS TREATED WITH ADJUVANT CHEMOTHERAPY FOR RETINOBLASTOMA.

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A higher relative frequency of Retinoblastoma (RBL) has been reported in developing countries. This increase has also been observed at Baragwanath Hospital, and in addition an advanced stage of disease, at equivalent ages, is seen compared to developed countries. Since RBL appears to present more aggressively compared to developed countries we wished to evaluate whether the outcome of RBL patients, matched for stage, differed or was the same compared to developed countries. Local extension into the optic nerve was seen in 54% of patients and metastatic disease in 32%, making the use of ajuvant therapy essential. Staging of the disease in our centre was based histologically as surgery was often done upfront. Chemotherapy protocols consisted of ICE:Ifosphamide, Carboplatinum and Etopside (10 patients) and changed due to unacceptable toxicity to VEC:Vincristine, Etosposide and Carboplatinum (32 patients). Triple intra-thecal therapy and radiation treatment was offered depending on the stage of disease.
Using Kaplan-Meier curves for the study period 1989-1994
- ICE 20%(95% CI - 4.8%; 44.8%) EFS
- VEC 30%(95% CI - 12.8%; 72.8%) EFS
Stage 1- 87.5% (95% CI 64.6%; 110%) EFS
2- 26.5% (95% CI 1.7%; 51.5%) EFS
3- 14.4% (95& CI-4.2; 35%) EFS
4- 17.9% (95% CI-13.6%; 49.3%) EFS
Unilateral - 43.2% (95% CI 26.5%; 60.0%) EFS and Bilateral - 0% EFS.
Outcome is worse for all stages in our study group probably because of advanced stage at presentation, or more aggressive or perhaps more resistant disease. Early detection is crucial in the outcome of patients with RBL, but adjuvant therapy dose have an important role to play in the treatment of sight preservation and in invasive disease. More data is required to help address the important issues surrounding this potentially curable malignant disease.

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REGULATION OF APOPTOSIS IN RHABDOMYOSARCOMA BY PAIRED DOMAIN TRANSCRIPTION FACTORS

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Alveolar rhabdomyosarcoma (RMS) is characterized by a specific translocation t(2;13)(q35;q14) generating a PAX3/FKHR fusion gene. This gene encodes a chimeric transcription factor involving the DNA binding domains of the paired class transcription factor Pax3 and the transactivation domain of a fork head domain gene, FKHR. The specific occurrence of the fusion protein in this type of cancer suggests a causal role in the generation of this tumor.
To obtain direct evidence in support of this hypothesis, we developed an antisense oligonucleotide strategy designed to downregulate the fusion protein in a RMS cell line (Rh30). Our results demonstrate that incubation of this translocation carrying cell line with antisense oligonucleotides leads to a concentration dependent reduction in cell number and cell death. This effect was specific since it was neither observed with control oligonucleotides nor in fibroblast cells not expressing Pax3. Interestingly, in cell lines representing the more prevalent subtype embryonal RMS (RD and Rh1), native Pax3 protein and/or its close homologue Pax7 are found at elevated levels, but no translocation is present. Again, cell death could be induced in these cell lines with appropriate oligonucleotides. Further experiments demonstrated that cell death was due to apoptosis and not necrosis. Hence, our results demonstrate that paired domain transcription factors are involved in the regulation of apoptosis in tumor cells. This suggests that treatment based on the inhibition of Pax3/Pax7 function represents an attractive new avenue in this type of sarcoma.
Therefore, from a clinical point of view, our results imply that an overexpression of Pax proteins might represent a common feature in RMS. To assess the importance of this observation in vivo, we are screening archieved tumor material for expression of the native Pax proteins as well as the PAX/FKHR fusion proteins. The results of this study are compared to clinical outcome and should reveal the significance of these proteins for prognosis.

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CLINICAL RELEVANCE OF DNA PLOIDY AND S-PHASE FRACTION IN CHILDHOOD RHABDOMYOSARCOMA (ENROLLED INTO THE ITALIAN COOPERATIVE SARCOMA STUDY-ICS RMS 88)

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DNA content has been indicated as a potential prognostic factor for many childhood malignancies. In order to evaluate the possible prognostic relevance of DNA ploidy and proliferative activity (SPF) in childhood rhabdomyosarcoma (RMS), we conducted a retrospective study on 59 RMS cases entered the ICS-RMS88 protocol (7 RMS botryoid, 35 embryonal and 17 alveolar RMSs), for whom formalin-fixed paraffin-embedded tissue is available. From each sample a 5μm section was cut for histological control. Nuclear suspenction for cytometric investigations was obtained using a mechanical disgregator (Medimachine, Consult-TS). With this method the variation coefficient had a mean value of 4% (range 2.6% - 6.3%). The DNA histograms were diploid ($0.9<DI<1.1$) in nineteen (33%) cases, hyperdiploid ($1.1\leq DI<1.8$) in twenty nine (49%), tetraploid ($1.8\leq DI<2.2$) in ten (17%), and multiploid in one case. The 5-years overall survival (OS) by ploidy status were: hyperdiploid 73%; diploid 33% and tetraploid 25% ($p=0.0012$). a striking difference emerged comparing the 5-years OS of the diploid and tetraploid RMS combined, with the one of the hyperdiploid RMS alone: 73% and 30% respectively ($p=0.0006$). With a cut off of 14% even SPF resulted prognostically relevant; in fact 5 years OS by SPF less or more than 14% were 70% and 36% respectively ($p=0.009$). In a multivariate analysis including stage, anatomic site, histologic subtypes, only DNA content ($p=0.0006$) and SPF ($p=0.034$) turned out to be able to influence significantly OS. In conclusion: the relevance of these important findings should be kept in mind in plaining future clinical trials and on childhood RMS.

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EVALUATION OF THREE DOUBLET CHEMOTHERAPY REGIMENS IN METASTATIC RHABDOMYOSARCOMA IN IRSG-IV

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With the objective of identifying an effective new doublet chemotherapy regimen, the Intergroup Rhabdomyosarcoma Study Group (IRSG) Committee undertook an evaluation of Vincristine/Melphalan (VM), Ifosfamide/Etoposide (IE), and Ifosfamide/Doxorubicin (ID) in patients with metastatic rhabdomyosarcoma. In this trial 152 patients were placed on ID and 126 were randomized between VM and IE. The more recently completed randomized portion of the study closed March 1, 1995. The complete response rate for each of the three doublets was 38% (25/65) for VM, 39% (24/61) for IE, and 49% (74/152) for ID. Patients on the ID regimen had been followed for a significantly longer period of time which may account for the increased response rate. The toxic death rate was not significantly different in any of the three regimens, with IE being 1.8%, VM 3.4%, and ID 5.9%. Infection was the cause of toxic death in 8 of 13 cases. One patient with VM expired of veno-occlusive disease (VOD) of the liver after receiving VAC chemotherapy in the maintenance phase of the trial. Progression free survival (PFS) at one year for 65 patients eligible and randomized on VM was 56% (+ 7%). For 61 eligible and randomized patients on IE the PFS was 68% (+ 6%). For 152 non-randomized patients on ID, the PFS was 61% (+ 4%). The PFS between the three doublets was not significantly different with p=0.24. The overall survival at one year for VM, IE and ID was 72%, 78%, and 78% respectively with p=0.11.

DOUBLET	TOXIC DEATH RATE	CR RATE	SURVIVAL 1 YR	PFS 1 YR
VM	3.4%	38%	72%	56%
IE	1.8%	39%	78%	68%
ID	5.9%	49%	78%	61%

Based on the lowest toxic death rate and best PFS at one year, IE would appear to be the best doublet regimen for incorporation into future multi-agent trials in children and adolescents with rhabdomyosarcoma.

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AN ANALYSIS OF PROGNOSTIC FACTORS AND COMBINE MODALITY TREATMENT IN CHILDHOOD RHABDOMYOSARCOMA

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During the years(yrs) of 1972-1992, 255 previously untreated patients(pts) with rhabdomyosarcoma (RMS) (age ranged between 15 days to 17 yrs with a median 5 yrs) were evaluated and treated in our institution. Head and neck primaries were seen in 125 pts(49%), abdomino-pelvic in 73(29%), trunk and lung in 20(5%) and extremity lesions in 37(15%). The histology was; embryonal,137; alveolar, 42; botryoid,18; pleomorphic, 14; and could not be subclassified in 42 pts. All work ups including, chest-X ray, rutine blood and biochemical tests. Further studies had been done like cerebrospinal fluid examination and CT scan depended on the primary site. The stage of the pts were as follows; 15 in stage I, 74 in stage II, 139 in stage III and 27 in stage IV according to IRS grouping system. Patients were treated with various combination of surgery (excision or debulking), radiation to doses of 35-55 Gy according to the patients' age and stage. All the pts received chemotherapy according to VAC or PULSE-VAC (before 1988) and modified AVAC (after 1988) protocol. Survival curves were calculated by the Kaplan-Meier method. The stastical significance of each variable was tested by the log-rank test. Overall survival was 42% at 10 yrs. Histology and sex were not related to survival. Three important predictors for survival time were clinical group ($p<0.001$), age ($p<0.001$) and primary site ($p=0.005$). The best results involved; clinical group I-II, age1-5 yrs and orbital, genitourynary primary sites. There was also detected an important predictor for survival time between first ten yrs(1972-82) and last 10 yrs (1982-92) $p<0.005$. Of the 96 deaths, 24 were from unknown causes, 35 to septicemia related to the therapy protocols. The overall results of this trial represent a significant improvement over our own institution's in last ten years.

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IMMUNOHISTOCHEMICAL DETECTION OF p53 PROTEIN IN RHABDOMYOSARCOMA AND ASSOCIATION WITH CLINICOPATHOLOGIC FEATURES

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Background: Alterations in the p53 tumor suppressor gene are the most common tumor specific genetic changes, having been identified in most major cancer types, including rhabdomyosarcomas. The stabilization or conformational change of wild-type p53 protein may lead to an important increase in its half life and thereby result in its accumulation in the nucleus of the tumor cells, which can be detectable by immunocyto-chemical methods.
Methods: Formalin-fixed paraffin embedded tissue sections obtained from 31 cases of rhabdomyosarcoma were immunostained with a mouse monoclonal antibody p53-D07, and a relationship between clinicopathologic features and the overexpression of p53 protein was investigated. Staining was assessed by evaluating the percentage of p53 immunopositive cancer cell nuclei and by the degree of the intensity of staining.
Results: Nuclear accumulation of p53 protein was detected in 4 of 31 (13%) samples. Clinical analyses of patients demonstrated an association between positive staining and advanced stage, progressive or recurrent disease and decreased survival.
Coclusion: The nuclear p53 immunoreaction rate is not very high in rhabdomyosarcoma but a correlation between nuclear staining and the tumor aggressiveness is suggested.

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PROGNOSTIC VALUE OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) IMMUNOSTAINING IN PEDIATRIC RHABDOMYOSARCOMAS

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The levels of proliferating cell nuclear antigen (PCNA) are almost negligible in long-term quiescent cells and increase dramatically during the cell cycle. Recently, the monoclonal antibodies to PCNA has been used to demonstrate the proliferative component of paraffin embedded tumor tissues. It has been shown to be available as a simple histologic marker of proliferative activity and the PCNA labeling index has been correlated with the prognosis of several malignant neoplasms. Formalin-fixed, paraffin embedded tissue specimens of 17 primary pediatric rhabdomyosarcomas were immunostained using an anti-PCNA monoclonal antibody. Then, the relationship between the PCNA index, prognosis, and various other factors were assessed retrospectively. The mean PCNA index was 53%. Patients having tumors with a high PCNA index (>53%) showed lower survival rates than tumors with a low PCNA index ($p<0.0005$). Moreover, there were significantly more relapsing or progressing cases in the high PCNA index group ($p<0.001$). As a conclusion, the PCNA labeling index was shown to be a useful indicator of recurrence and survival in pediatric rhabdomyosarcomas.

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RABDOMYOSARCOMA (RMS) ARISING FROM 2 RARE PAEDIATRIC TUMORS WITH HETEROLOGOUS ELEMENTS.

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RMS, has rarely been described as the end step of a transformation of tumours containing mesenhymal or heterologous rhabdomyomatous elements. We present 2 different very rare tumours, a sex-cord stromal tumour of the ovary (Sertoli-Leydig cell tumour with retiform pattern SLCT-RP) and a pulmonary blastoma of the lung, that both evoluted to RMS. **The first case** was diagnosed in a 10 year old girl, presenting with acute abdomen. At operation a 17cm multicystic tumour of the right ovary in torsion, was found and right oophorectomy was performed. The tumour was initially thought to be an endodermal sinus tumour but finally it was diagnosed as a well differentiated SLCT-RP with small foci of embryonal mesenchyme. Three months later the patient recurred with a 15cm edematous ruptured tumour with histological features of embryonal RMS which arised from heterologous rhabdomyoblastic elements of the original SLCT-RP. **The second case** was a 22 month old girl who presented with repeated episodes of pneumothorax. At explorative thoracotomy, a tumour located at the lower lobe of the right lung, measuring 4x4x2cm was found and segmentectomy was performed. The tumour had a multi-cystic appearance and soft tissue consistency. Microscopically the cysts were lined by a layer of columnar or cuboidal cells. The intervening cells were small with scanty cytoplasm, many mitoses and a tendency for crowding beneath the epithelial lining, giving the impression of "cambium like" arrangement. Nine months later recurrence of a sarcomatous tumour occurred with cells displaying intense pleomorphism, multinucleated giant cells, having moderate amount of eosinophilic cytoplasm and striations in several of them. The tumour was completely resected with lateral lobectomy. Both patients received a combination chemotherapy and local radiation therapy and both remain in complete remission after 18 and 20 months respectively. The rarity of RMS originating from SLCT-RP and pulmonary blastoma renders the understanding of their course and prognosis difficult. Further observations on similar cases may provide a better understanding of their evolution and outcome.

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SOFT TISSUE SARCOMAS (STS) OF THE HAND AND FOOT

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Of 550 patients (pts) with STS treated over the past 30 years, 36 (6.5%) had a primary tumor in the hand or foot. The median age of the 13 nonrhabdomyosarcoma (NRSTS) and 5 rhabdomyosarcoma (RMS) with foot primaries was 13.5 yrs compared to 11 yrs for the 7 NRSTS and 11 RMS arising in the hand. In all pts mass was the most common presentation, but in foot primaries pain was the most frequent complaint. Metastatic disease was noted in 12/16 (75%) pts with RMS compared to only 3/20 (15%) NRSTS pts. In pts with RMS alveolar histology was seen in 11/16 (69%). For NRSTS, synovial sarcoma accounted for 40% of cases. All 5 pts with foot RMS had disseminated disease at diagnosis and none survive. Only 3/13 (23%) pts with NRSTS had metastasis at diagnosis. Most of localized NRSTS presented with low grade noninvasive lesions. Only one had a below-knee amputation. All others had wide local excision (WLE) or ray amputation (RA). 7/10 (70%) survive (median 7 yrs). All 7/11 with hand RMS who had metastatic disease at diagnosis have died. Of the children with localized RMS 3/4 survive following chemotherapy, local excision and RT. All 7 pts with hand NRSTS had localized disease. Treatment consisted of WLE (3), RA (3), and supplemental RT (3). 6/7 (85%) survive. All surviving pts have excellent function. We conclude: 1) Patients with hand and foot RMS more commonly present with disseminated disease compared to NRSTS. 2) For localized disease conservative surgery, WLE, or RA ± RT results in cure rate of 80% with good function.

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MESENCHYMAL CHONDROSARCOMA IN CHILDREN AND ADOLESCENTS: A REPORT OF 11 CASES FROM THE GPOH-CWS STUDY GROUP.

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Mesenchymal Chondrosarcoma (MCS) is a very rare tumour in children and adolescents. Since the start of the German Cooperative Soft Tissue Sarcoma Studies (CWS) in 1981, only 11/1425 patients (0.8%) with chondrosarcoma have been enrolled to the protocols CWS-81/86/91 and 96P. The major distinction between mesenchymal and other chondrosarcomas is the objective soft tissue component influencing treatment and prognosis (high malignancy, more metastasis).

Age	Sex	Site	Size	T-	NM-Stat.
range: 8-25 yrs.	fem.: 9	head/neck	< 3 cm: 1	T1a: 2	N0: 11
median: 15 yrs.	male: 2	- pm.: 4	3-5 cm: 1	T1b: 1	N1: -
		- npm: 1	6-10 cm: 5	T2a: -	
		- orbit: 1	> 10 cm: 3	T2b: 7	M0: 11
		limbs: 3	n.e.: 1	n.e.: 1	M1: -
		paravertebral: 2			

***Table 1: Patients characteristics** of 11 pts. with MCS* (n.e.=not evaluable)

Initial non-mutilating tumour resection was attempted in 9 cases, while 2 patients had only been biopsied initially. In 3 cases a microscopical complete excision of the tumour was achievable (pT1 & pT2), in 3 cases only a marginal tumour resection was possible (pT3a) and in 5 cases a gross residual tumour had to be left in the tumour bed (pT3b).

Postsurgical state	pT1/2	pT3a	pT3b	total
n	3	3	5	11
evaluable (f/u > 2 yrs.)	3	3	3 *)	9
Chemotherapy Response	1 (VAIA) n.e.	3 (2xVAIA, VACA) 1 prog., 2 n.e.	3 (2xVAIA, EVAIA) 1 nr, 1 prog., 1 n.e.	7
RTX	-	1	1	2
Second resection	-	1	3 (2 mutilating)	2
relapse	-	1 (local)	-	1
alive in CCR	3 (100%)	2 (66%)	3 (100%)	8
dead	-	1 (33%)	-	1
Follow-up	3.5, 4, 5 yrs.	3, 6 yrs.	2.5, 4 and 6 yrs.	4 yrs.

Table 2: Treament and outcome (n.e.: not evaluable, nr: non-responder, f/u: follow up, prog.: tumour progression *) 2 pts. n.e.: 1 with 3 months f/u, uncertain therapy, 1 with ongoing therapy)

In summary 8/9 patients (89 %) with MCS (follow-up > 2 yrs., median 4 yrs.) are alive in CCR. While these numbers are far too low to state clear recommendations, the role of chemotherapy seems widely unproven. Surgical complete removal of the tumour, however, is the most promising element in the treatment of MCS, while initial mutilating surgery seems to be unnecessary because especially in high-grade MCS radiotherapy with/without chemotherapy in combination with primary or secondary marginal resection may be sufficient in providing local control.

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NON-RHABDOMYOSARCOMA SOFT TISSUE SARCOMAS: CLINICAL CHARACTERISTICS AND OUTCOME IN 42 CHILDREN OVER 18 YEARS AT THE DENVER CHILDREN'S HOSPITAL

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Non-rhabdomyosarcoma soft tissue sarcomas (NRSTS) account for nearly 50% of all pediatric soft tissue sarcomas seen in the USA. Unlike rhabdomyosarcoma, there have been few formal clinical trials to evaluate therapy and outcome in this diverse group of tumors. Treatment for these children has usually been individualized. We report here the experience at one institution with NRSTS over an 18 year period. Forty nine patients had tumors with the diagnosis of NRSTS between 11/77 and 1/96;charts on 42 patients were available for review. The most common type of NRSTS diagnosed was fibrosarcoma (26%) followed by synovial cell sarcoma (14%), malignant schwannoma (12%), and malignant mesenchymoma (12%). The median age at presentation was 6 years 4 months (range-birth to 20 years 4 months). The M:F ratio was 1:1.6. Sixty-four percent of patients had stage I/II disease and 36% had stage III/IV disease. Fifty percent of patients with stage I disease were downstaged by further initial surgery. Sites of disease included extremities (45%), head/neck (24%), and abdomen/pelvis (17%). Chemotherapy was administered to 64% of patients. VAC and/or VAdrC were used in almost 80% of children receiving chemotherapy. Cisplatin, etoposide, DTIC, and methotrexate were used less frequently. Radiation therapy was used in 17/42 patients (40%) including 4 children who received intraoperative radiation. The radiation dose range was 1000-6000cGy. Surgery alone was used to treat 15/42 patients (36%). The relapse free survival (RFS) and overall survival (OS) at 6 years are 58% and 63% respectively. For patients with stage I/II disease, the RFS is 76% and OS is 80%. For stage III/IV disease the RFS is 37% and OS is 32%. Our findings indicate good survival for patients with low stage disease which make up the majority of patients with NRSTS in this study. As most patients with this disease are not treated in a uniform fashion, cooperative studies should be encouraged in order to determine the best approach to therapy.

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LOCALIZED PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMORS (PPNET) AND EXTRAOSSEUS EWING'S SARCOMAS (EOE): The experience of the Italian Cooperative Study (ICS) RMS-88

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PPNET and EOE belong to a family of aggressive sarcomas with similar histogenetic origin and clinical features. From January 1988 to June 1995 38 evaluable pts with histologically proven localized PPNET(20) and EOE(18) were registered in the ICS RMS-88. **Clinical features.** PPNET: 11 M and 9 F, median age 104.5 mos (range 5-199); primary sites: head-neck 2, parameningeal 1, extremities 4, others 13. EOE: 12 M and 6 F, median age 142.2 mos (range 6-190); primary sites: head-neck 1, parameningeal 1, extremities 7, others 9. **Treatment.** All pts were classified according to the IRS system and treated as follows: GR.I (complete resection) chemotherapy (CT) according to IVA regimen (3 cycles); GR.II (microscopic residuals and/or nodes involved): CT (IVA 3 cycles) and radiotherapy (RT-40Gy); GR.III (biopsy or macroscopic residuals): CT (VAIA regimen) followed by delayed surgery, CT (IVA 3 cycles) ± RT (40 or 54 Gy). **Results**, (follow-up 6-80 mos.). PPNET. 3 GR.I pts are alive without disease (NED); 1 GR.II pt had local relapse (LR) and died 6 mos from diagnosis; among 16 GR.III pts, 9 are alive NED, 2 are alive with disease after LR, 5 died: 2 for LR, 2 for progressive disease (PD), 1 for toxicity; EOE: 1 GR.I pt is alive NED; 2/3 GR.II pts are alive NED and 1 died (LR + lung metastases); among 14 GR.III pts, 8 are alive NED, 6 died (3 PD, 1 brain metastases, 2 LR). The 5-year OS and PFS are 68% and 72% for PPNET and 44% and 58.2% for EOE. **Comment.** In our series PPNET seems to have a better outcome. The most important cause of treatment failure was the inability to achieve or to maintain the local control in GR.II and GR.III pts (8 LR and 5 PD) even if 10 out of 13 pts recived RT. In these patients more aggressive surgery, or pre-operative RT may be considered in order to improve the local control.

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MODERATE CHEMOTHERAPY AMPLIFIED BY REGIONAL HYPERTHEMIA FOR RECURRENT, PROGRESSIVE OR UNRESECTABLE DESMOID FIBRMATOSIS IN CHILDREN.

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Introduction: Desmoid or aggressive fibromatosis are benign (myo)-fibroblastic tumors, that characteristically tend to destructive infiltration of the adjacent tissue and can cause severe morbidity. The mainstay of treatment is surgical removal, but local recurrence and functional impairment after mutilating surgery are frequent problems. Additional or alternative local irradiation and systemic chemotherapy has been reported. However these only occasionally successful but aggressive therapies are hard to justify in children and adolescents for what are essentially considered benign lesions. For that reason we tried to intensify a moderate chemotherapy by hyperthermia to treat recurrent, progressive or unresectable desmoid fibromatosis.

Patients: We report on six children from 9 months to 16 years of age with relapsed, progressive or inoperable desmoid fibromatosis. All patients had received multimodal pretreatment containing multiple surgical resections (5), radiotherapy (3) and chemotherapy (2). The aim of our therapy with moderate chemotherapy and rHT was to achieve a stagnation of tumor growth and to avoid surgical mutilation. Four patients presented with extra-abdominal fibromatosis of the limb; in two of them a hemipelvectomy was considered before rHT. Two patients had a extended intra-abdominal fibromatosis.

Treatment: Regional heating was delivered with an electromagnetic heating device (BSD2000) at 70-200 MHz with external applicators and energy levels up to 2000 Watt. The desired intratumoral temperature was 42.5 ° Celsius for 60 minutes. Chemotherapy consisted in carboplatin during the rHT combined with vincristine (3), dacarbacin (1), etoposide (1) or etoposide + vincristine (1) directly before. An average of 10 treatments (6-13) per patient were performend.

Outcome:. Tumor control was achieved in five patients, mutilating surgery could be avoided. One patient had a size reduction of 64% of the intra-abdominal tumor. Four had a stagnation of tumor growth. One boy had progressive disease during treatment. After subsequent radiation his tumor decreased. The progress free survival is 42, 30, 5 and 2.5 months. One patient is still under treatment.

Conclusion: In children with progressive and inoperable fibromatosis a stagnation of tumor growth can be achieved with a combination of moderate chemotherapy and rHT. More patients and longer observation are needed to assess the potency of this new treatment modality.

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EARLY TREATMENT RESULTS OF 26 CHILDREN WITH SOFT TISSUE SARCOMAS OBTAINED DURING 1991 - 1994 ACCORDING TO OWN PROTOCOL

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The analysis of treatment results obtained in 26 children with soft tissue sarcomas (STS)according to the main clinical characteristics as possible prognostic factors was performed.
During 1991-94 26 children with STS, 17 boys and 9 girls aged 10 mos to 15 yrs (median 6 yrs, the majority with very advanced disease (73% in III and IV groups of IRS classification)were treated according to our own protocol.
In RMS the regimen VACA/VAIA, in non-RMS CYVADIC were used.
In both IV groups induction CHT consisted of 5-drugs regimen IRS-III. Poor previous results in extremity location and alveolar RMS caused their upgrading to IV group independently of clinical advancement. 17 patients (pts)survived with mean follow up 29 mos, in RMS - 10 (71,4%), in non-RMS - 7(58,3%). The better results in children < 5 yrs and older than 10 yrs in comparison with 5-10 yrs were only statistically proven (p=0,012). The other results were NS because of small number of pts in the compared groups but all the same they are convincing. In group I and II of clinical advancement all 7 pts are alive, in III and IV 10 from 19(52,6%). In urogenital locations 85,7% survived, in unfavorable locations together 57,9%. Poor results were obtained in parameningeal (25%). The improvement in extremity location (75%)and alveolar RMS (66,6%)proved that our decision of their upgrading was reasonable. The influence of sex (better results in girls), tumor size (<5cm and >5cm respectively 75% vs 46,7%)and type of remission (CR - 80%, PR - 46,2%)were also observed.
The main unfavorable factors should cause upgrading in STS classification thus intensifying the treatment.

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AUTOGRAFT BONE TRANSPLANTATION - AN ALTERANATIVE FOR LIMB SALVAGE PROCEDURES IN CHILDREN.

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Limb sparring procedure can be considered for treatment of malignant bone tumors. Conventional procedures include resection of expendable bone and substitution by endoprostesis or allograft bone transplantation for non expandable bone. Autogenous non vascularized graft can be used in the substitution of non expendable bone (femur, fibula and umerus).
Between 1984 and 1992, 20 patients with malignant bone tumor (9 osteosarcomas and 11 Ewing's sarcomas) were clincally good responders to pre-operatory chemotherapy underwent this surgical procedure. Age ranged between 3 to 17 years (mean 7y). Primary site was tibia in 10 patients, femur in 8 and umerus in two. 19 patients received fibula graft plus iliac chips and extramedular rod.and one only iliac chips and extramedular rod. Growth cartilage could be preserved in all patients.The most frequent complication was non union in 5 patients (25%) fatigue fracture in 4 patients (20%) and infection in 2 patients (10%). Although early complication was common late results showed in 18 patients solid union and extremity growth could be preserved.
In summary, this procedure is an alternative for limb salvage procedure in selected patients where amputation due to the age is indicated.

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RECONSTRUCTING BONE TUMOR RESECTION IN CHILDREN BY VASCULARIZED FIBULAR TRANSFER.

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Eleven children underwent extensive bone resection (10 cm to 24 cm) with immediate or secondary bone reconstruction by vascularized fibular transfer. Ewing sarcoma was diagnosed in 6 cases, osteosarcoma in 5 cases, and Carney complex in 1 case. Bone tumor concerned 5 tibias, 5 femurs, and 1 radius. Wide local excision was used in all cases. Immediate reconstruction was conducted in 9 cases. In 1 case reconstruction was delayed to assess the resection margins. In another case the vascularized transfer was performed after the non-vascularized bone graft failure. Free transfer was used in 9 cases and pedicled transfer in 2 cases. Fibula were reconstructed by autogenous cortical graft to prevent growth deformation of the donor leg. Shaft stabilization was provided by either internal or external fixation. Proximal and distal stump of the fibular transfer were stabilized in lay with or without pins. The mean follow up was 4.9 years. Bone healing was achieved in all except 1 case, but additional cancellous bone graft was needed in 5 cases to insure proximal or distal union of the transfer. Vascularized bone transfer hypertrophy was observed within 2 years after surgery. Ten patients gain normal life with regular sport training. One patient suffered a local tumor recurrence with metastases. We conclude that vascularized fibular transfer is a reliable procedure, in children, to reconstruct bone tumor resection with respect to carcinologic excision.

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INTENSIVE CHEMOTHERAPY (CT) WITHOUT IRRADIATION (XRT) FOR POOR PROGNOSIS WILMS' TUMOR (WT)

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Five consecutive newly diagnosed children with metastatic or unfavorable histology WT were treated with a time-intensive CT regimen. Patients (pts) were ages 2.5, 4, 4, 6 and 15 years. Three pts had favorable histology stage 4, one had focal anaplasia stage 4 and one had diffuse anaplasia stage 3 with anaplasia in lymph nodes. All stage 4 pts had multiple bilateral lung metastases by chest x-ray and CT scan. All pts underwent nephrectomy at diagnosis. NWTS would have required whole lung XRT in 4 of 5 and abdominal XRT in 3 of the 5 pts. No XRT was used in this regimen. The CT consisted of induction, consolidation and maintenance phases. Induction included doxorubicin 50 mg/M^2 and cyclophosphamide 1 gm/M^2 on days 0, 14 and 35. Etoposide 300 mg/M^2 over 3d and cisplatinum 100 mg/M^2 over 3d were given on days 1-3, 15-17 and 36-38. The day 14 cycle was given regardless of blood counts. Consolidation therapy consisted of the same drugs and doses given at 4-week intervals with marrow recovery required. During maintenance therapy, doxorubicin and cyclophosphamide alternated with etoposide and cisplatinum every 4 weeks. The total planned dose of doxorubicin was 450 mg/M^2. GCSF was given to 4 of 5 pts. Dose limiting toxicity was myelosuppression. There were no grade 3 or 4 cardiac, renal or auditory toxicities. Four of 5 pts are complete responders. One patient, a partial responder radiographically, continues to show decrease in tumor volume and is still on therapy 8 months post diagnosis. The other 4 pts are off therapy and free of disease 15+, 18+, 22+ and 64+ months from diagnosis. The therapy was given as an outpatient in the last 4 cases. The treatment appears effective and warrants further study.

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ULTRASOUND GUIDED CUTTING NEEDLE BIOPSIES IN PEDIATRIC RENAL AND JUXTARENAL TUMORS

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In 1988, we introduced ultrasound guided, cutting needle biopsies (Ø = 1.2 mm) during the work-up of patients presenting with renal and juxtarenal (i.e. a tumor continuous with the kidney tissue) tumors for a pretreatment histologic diagnosis. The aims of this study are to determine the safety and clinical value of such cutting needle biopsies. The Akademiska Hospital´s material of renal and juxtarenal tumors was reviewed, and in 21 consequtive patients such a biopsy had been performed under general anaesthesia. The patients´ hospital records were reviewed and updated, and the histologic slides from the biopsies and the nephrectomy specimens were re-examined in a blinded fashion by a SIOP-panel expert pathologist (B.S.). No biopsy complications — such as tumor seeding at surgery or at follow-up, fall in hemoglobin concentration or anaesthesiological complications — were observed in our material. The clinical value, expressed as the concordance between the initial biopsy and the nephrectomy specimen, was 76% (16/21), since biopsies in 5 patients rendered material that was not diagnostic. The diagnoses were: Wilms´ tumor (13 patients; one of which had anaplasia), malignant rhabdoid tumor of the kidney (2 patients), neuroblastoma (2 patients), mesoblastic nephroma (2 patients), clear cell sarcoma (1 patient) and adenocarcinoma (1 patient). Of the Wilms´ tumor cases, 9 were stage I, 1 stage III, 1 stage IV, and 2 stage V. In conclusion, ultrasound guided cutting needle biopsies in pediatric renal and juxtarenal tumors was a safe procedure which rendered a histological diagnosis prior to treatment in 16 of our 21 patients.

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WILMS' TUMOR: IDENTIFICATION AND MAPPING OF NEW TUMOR SUPPRESSOR GENE(S) TO CHROMOSOME 7p

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At least two different genes (WT1 and WT2, both mapped to chromosome 11p) have been so far reported to be involved in the early stages of Wilms' tumor. However, in a large proportion of cases (50-70%), no alterations affecting these genes can be demonstrated, thus suggesting the presence of other loci associated with this neoplasia. Kariotypic investigation of 11 cases revealed frequent rearrangements involving chromosomes 1,7,11 and 16. While previous studies have suggested that mutations of genes mapped to chromosomes 1 and 16 are likely to be associated with the progression of the tumor, no molecular data concerning a role for 7p genes in this pathology were available. To test this hypothesis, we performed molecular studies on a larger group of tumors (38 cases) using microsatellite markers of chromosome 7p. This investigation revealed loss of heterozygosity or imbalance for 7p markers in 9 out of 36 informative cases (25%), and also permitted to better define the position of gene(s) involved in Wilms' tumor within an interval of approximately 25cM. No preferential parental origin of the lost allele was observed, thus excluding a genetic imprinting mechanism affecting chromosome 7p losses.

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NEPHROBLASTOMA IN CHILDREN AGED LESS THAN 6 MONTHS AT DIAGNOSIS - A REPORT FROM THE POLISH WILMS TUMOR STUDY.

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Nephroblastoma in newborns and infants seems less aggressive and has better prognosis than in older children. Aim: to evaluate incidence, staging and results of treatment of the infantile nephroblastoma. The First Polish Wilms Tumor Study has registered 147 patients (1993-95). Twelve of them were aged less than 6 months (8%): 5 were newborns, 7 - infants. Tumors were right sided in 8 and left - in 4. Protocol was based on the current SIOP 93-01 scheme and suggested primary nephrectomy in pts aged less than 6 months. Postoperative treatment consisted of VCR-ACTD +/- EPI +/- radiotherapy and depended on stage and histology. Doses of drugs were reduced to 2/3. All pts were operated on primarily. Tumor-nephrectomy was radical in 7 (stage I in 5, IIN^0 in 2) and irradical in 5 pts (stage IIN1 and III). Histology was of low risk in 4 pts (stage I/3, IINO/1), intermediate risk in 6 (stage I/2, IIN-/1, IIN+ &III/3) and high risk in 2 (both stage III). Postoperative treatment consisted of VCR-ACTD regimen in stages I and II and VCR/ACTD/Epirubicine in stages IIN+/III. Because of doubt in diagnosis of high risk histology, the standard risk protocol has been given to these pts. Low risk stage I patients were not treated postoperatively. Outcome: follow up ranges from 10 to 32 months (median 31). Eleven pts are in the first CR. One died of tumor. As surgical complications, 2 tumor ruptures, and 1 small bowel intussusception were registered. Chemotherapy tolerance was acceptable (below grade III of toxicity scores). Conclusion: Predominance of stage I has not been observed, however the favourable prognosis of nephroblastoma in early age was confirmed. Postoperative treatment was well tolerated. (KBN grant 4S405-075-07).

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INTENSIVE INDUCTION CHEMOTHERAPY (CT) FOR RECURRENT OR UNRESPONSIVE WILMS' TUMOR (WT): A SINGLE INSTITUTION STUDY.

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A total of 22 patients (pts) with recurrent or unresponsive WT were treated with two courses of Etoposide (VP-16), Carboplatin (Car), and Cyclophosphamide (Cy) / or Ifosfamide (Ifo). All pts belonged to high risk group (III or IU initial stage in 18 pts, prior CT with 3 or more agents in 20 pts, prior radiation therapy in 10 pts, diagnosis to relapse interval < 6 months in 6 pts, and unfavorable histology in 5 pts). The drug regimen consisted of VP-16 (100 mg/m2/d x 5), Car (100 mg/m2/d x 5), Cy (400 mg/m2/d x 5) / or Ifo (1800 mg/m2/d x 5) with MESNA. GM-CSF was administered in 7 pts. Evaluability was limited in 7 patients due to complete resection prior CT or for lack of imaging. Among 15 evaluable pts, there were 4 complete responses, 8 partial responses, 1 with stable disease, and 2 with progressive disease. The overall response rate after two courses was 80%. After response evaluation, further treatment consisted of radiation therapy and/or surgery, depending on the tumor response and site, followed by ABMT (in 5 pts) or two additional courses of the same CT as consolidation. In all pts, a total of 61 courses of intensive CT were given. Interval between courses was 14 - 40 days with median of 20 days. The main toxicities found were leukopenia and thrombocytopenia. Fever and neutropenia occurred 38 times, infections 10 times. One patient died of Gram-negative sepsis. No severe nephrotoxicity occurred. The administration of GM-CSF decreased the median duration of leukopenia < 500/ml from 8.9 to 5.5 days ($p<0.05$), but did not reduce the occurrence of febrile neutropenia. Nine of 22 pts are alive and disease-free 2 to 30 months after completion of the therapy (median - 9.5 months). The response rate was encouraging with the acceptable toxicity. A comparison of Cy and Ifo in equitoxic doses will be performed with more pts entered on the study.

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HEPATOPATHY-THROMBOCYTOPENIA SYNDROME AFTER TREATMENT WITH ACTINOMYCIN D FOR WILMS' TUMOUR

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Introduction: The association of veno-occlusive disease of the liver and severe thrombocytopenia in children receiving Actinomycin D therapy for Wilms' Tumour has been termed the hepatopathy-thrombocytopenia syndrome(HTS). A review of our experience suggests that HTS resolves quickly with supportive care alone and Actinomycin D can be safely reintroduced in a modified regimen after resolution of HTS. *Patients and Methods:* Eight consecutive patients received preoperative chemotherapy consisting of Vincristine(1.5mg/m^2) iv bolus weekly and Actinomycin D (1.35mg/m^2) iv bolus second weekly for a planned total of six to eight weeks. Liver biopsies were performed on affected patients two to four weeks after the onset of HTS. *Results:* Four of eight patients with Wilms' tumour developed HTS seven to nine days after their second, third or fourth doses of Actinomycin D. In all four patients severe thrombocytopenia preceded the onset of liver function disturbance. Three of the four experienced subclinical liver function abnormalities prior to the onset of HTS. All patients recovered within eight to eleven days with supportive care. Each patient received three to six further modified doses of Actinomycin D post-operatively without recurrence of HTS.
Conclusions: Frequent bolus administration of Actinomycin D may be associated with a higher incidence of HTS. Unexplained and sudden onset thrombocytopenia may herald the development of HTS. In our experience Actinomycin D has been safely used in modified dosing schedules in patients experiencing HTS.

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A RETROSPECTIVE CLINICAL PATHOLOGICAL ANALYSIS IN PATIENTS WITH WILMS TUMOR TREATED IN ONE INSTITUTION

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Despite excellent prognosis for patients (pts)with Wilms tumor (WT)of favorable histology (FH)survival is still poor for patients with unfavorable (UH)one. To assess the incidence of FH and UH, the course of disease and final outcome in both groups, a retrospective analysis of patients treated in our Department was performed. Records and pathomorphology slides of 93 pts treated between Jan 1983 and Dec 1993 were reviewed. In all but 5 pts, under 6 mos of age, histology was evaluated after preoperative chemotherapy according to SIOP 5,6,9 protocols. FH and UH was defined according to Beckwith and Palmer classification Pathologic classification from primary FH to UH was changed in 1 pt. There were 70 pts (75,3%)with FH and 23 pts(24,7%) with UH. The survival was significantly better for pts with FH (91,4%)comparing to pts with UH (43,4%) (p=0,0001). Follow up ranged from 2-10,5 yrs (median over 6 yrs). Relapses were more frequent in pts with UH (60,8%)than in pts with FH(12,8%) (p<0,05). Both local and distant relapses were observed only in pts with UH. The cure rate of pts who relapsed was statisticaly higher for pts with FH (66,6%) comparing to those with UH(14,2%) (p=0,023). All deceased pts with UH died of progressive disease despite intensive treatment. Out of six deceased pts with FH 3 died of disease and 3 of treatment complications. The incidence of UH was higher in our pts comparing with data from NWTS and SIOP Although WT remains highly curable, UH tumors require new treatment modalities.

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WILMS' TUMOR (WT) IN IRANIAN CHILDREN : 20 YEARS FOLLOW-UP

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During the past 20 yrs we have treated 294 pts with proven WT , referred to our centers from all over the country. Relevant clinical data are as follow : M:F ratio 1:1 , age range 2 mths to 21 yrs, mean age 3.5 yrs and median age 3 yrs. Parental consanguity 30 pts ; 22 1st and 8 2nd grade cousins.Presenting symptoms:abdominal mass 260 pts (88.4%) and hematuria 34 pts (11.6%). Associated anomalies ; 8 pts (2.7%). Involved kidney : Rt 142 (48.3%) , Lt 152 (51.7%). both kidneys 7(2.4%). Standard staging : laboratory tests, imaging procedures , nephrectomy & histopathology . Stages at presentation : 57 stage I (19.4%),123 stage II (41.8%), 83 stage III (28.2%), 24 stage IV (8.2%),7 stage V (2.4%). Histopathology : FH 111 pts (37.8%) , UFH 183 (62.2%). Standard management comprised chemotherapy according to NWTS or SIOP protocols and radiotherapy to the tumor bed and sites of metastasis.
Overall results : 1) > 5 yrs FOD ; 97 pts (33%),2) < 5 yrs FOD and /or still under chemotherapy ; 67 pts (22.8%) 3) death due to PD and other complications; 54 pts (18.3%) 4) lost to follw-Up ; 76 pts (25.9%) .
Conclusion: the majority of our pts with WT presented with high stages and UFH.These factors being mainly responsible for their poor outcome and also for the high number of pts giving up the therapy . Poor socio-economic conditions , illitracy and inadequate medical attendance are other additional adverse factors affecting the outcome.

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SURGICAL APPROACH TO LUNG METASTASES IN CHILDREN WITH NEPHROBLASTOMA.

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From 1985 to 1994 we have registered 26 pts with st.IV. of nephroblastoma. All children were treated with pre-op chemotherapy based on the Nephro SIOP-6 protocol including VCR 1,5mg/m^2 (6x), ACT D 15µg/kg (9x), ADM 50mg/m^2(2x). After nephrectomy (d.42) in 16 pts plain X-ray and CT of the lungs were normal, in 8-pulmonary metastases were still detectable but operable, in 2 multiple, inoperable. Lung metastases were removed in 8 pts aged from 19 months to 9 years, 6 pts underwent wedge resections, 2 extended resections (lobectomy, segment ectomy). There was no operative mortality and no per and post-operative complications. All pts have received post-operative chemotherapy according to SIOP-6 or SIOP-9 protocol. 7/8 operated for pulmonary metastases achieved complete remission (CR). Follow up ranges from 12 months to 10 years. 6 children are in the first CR, one is alived with NED 7 years after lobectomy and segmentectomy, one died of tumor, one is still under maintenance therapy. We conclude that low morbidity and mortality after metastatectomy has led us to recommed this method of treatment in all pts when pulmonary metastases are still detectable and operable after pre-op chemo-therapy and post nephrectomy. Stappler technique is a good surgical procedure for removing periphery lung metastases of nephroblastoma.

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DRUG–RESISTANCE IN A NEUROBLASTOMA XENOGRAFT AFTER CYCLIC ADMINISTRATION OF CPT–11

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CPT–11, a camptothecin derivative with inhibitory activity of topoisomerase I, has a promising antitumor potential against human neuroblastoma(NB) in nude mouse xenograft study. However, NB xenografts, once decreased in size by administration of CPT–11 shortly showed more rapid regrowth compared with other antitumor agents currently used. Before the introduction of CPT–11 in clinical trial, it is required to elucidate whether cyclic administration of this agent induce earlier drug resistance. We also investigated whether the loss of sensitivity to CPT–11 has any correlation to the enzymatic activity of topoisomerase I in tumor tissues.

Materials and Methods: TNB9, a cytogenetically extremely malignant but chemo sensitive nude mouse xenograft of human NB was used in these experiment. CPT–11 at a dose of one third of mouse LD_{50} was administered intraperitoneally three times every 4 days to the nude mice bearing 100–200 mg of NB. After the tumor once regressed and then regrew, these treatments were repeated until tumor shrinkage with CPT–11 treatments was no more observed. The CPT–11 refractory tumors were then transplanted to other nude mice for evaluationof the consecutive identical treatments. Tissue topoisomerase I activities before and after treatment were assayed by relaxation of supercoiled plasmid DNA.

Results: NB xenografts turned completely–resistant to CPT–11 within 3 cycles of drug administration. However, CPT–11 resistant–NB xenografts restored drug sensitivity after passage to other nude mice, probably due to selective tumor take of sensitive fraction. There was no correlation between topoisomerase I activities in tumor tissues and drug sensitivity in these experiments.

Conclusion: CPT–11 might rapidly lose its antitumor activity to NB by cyclic administration. Topoiserase I activities of tumor tissue had no obvious correlation with drug sensitivity to CPT–11.

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CARBOXYPEPTIDASE-G2 RESCUE IN CHILDREN WITH ACUTE RENAL FAILURE FOLLOWING HIGH DOSE METHOTREXATE ADMINISTRATION

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Acute renal failure (ARF) following high dose methotrexate (HDMTX) is a life threatening condition. So far, no standard therapeutic approach has been established for its treatment. Carboxypeptidase-G2 ($CPDG_2$) is a bacterial enzyme that rapidly cleaves methotrexate (MTX) into inactive metabolites. We used $CPDG_2$ rescue in two children with ARF following HDMTX. Both patients received 1500 mg/3 hr leucovorin rescue and than due to the lack of MTX elimination, a single dose of 50 U/kg $CPDG_2$ i.v. bolus was administered without any adverse reaction. The first patient had osteosarcoma (OSC), received MTX at a dose of 12 g/m^2/6hr. $CPDG_2$ was administered at the 100th hour after starting HDMTX. The plasma MTX concentration measured by HPLC fell from 84.7 μM to 4.59 μM within 30 minutes and to 0.33 μM within 18 hours after administration of $CPDG_2$. Despite the effective reduction of MTX levels and continue thymidine rescue the previous long term exposure to MTX resulted in systemic irreversible toxicity including mucositis, acute worsening of renal function, pleural effusion, bone marrow suppression and septicemia. The patient died 23 days after the administration of $CPDG_2$. Second patient (ALL) was treated with 5 g/m^2/24hr MTX. Following $CPDG_2$ administration at 54th hr MTX level decreased from 7.63 to 0.19 μM within 30 min. In this case we used $CPDG_2$ without additional thymidine rescue. A temporary and reversible renal failure and cytopenia was detected, a complete recovery occurred by week 6th. Detailed pharmacokinetic analysis of MTX has been performed following the enzyme administration. Using a two-compartment open model $t_{1/2\alpha}$ = 0.035 hr and 0.008 hr, $t_{1/2\beta}$ = 12.665 hr and 0.674 hr has been calculated for the first and second patients, until the first 21 and 1.5 hr, respectively. After the enzyme treatment a second MTX peak occurred between 39-47th hr in both cases. Based on these experiences $CPDG_2$ appears to rapidly and significantly decrease plasma MTX concentrations and may prove beneficial in patients with MTX induced renal dysfunction. Careful plasma MTX guided leucovorin rescue after the administration of $CPDG_2$, evaluation for third space fluid and supportive therapy continue to be essential to successfully treat this medical emergency.

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CHILDHOOD HYPERSENSITIVITY TO ETOPOSIDE-CARBOPLATIN ASSOCIATION

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Recently, in addition to the well-documented hypersensitivity to etoposide (E), there have also been an increasing number of reports on hypersensitivity to carboplatin (CBDCA). In this study the allergic reactions to E-CBDCA association were described in children. The schedule of chemotherapy was E (300 mg/sqm in a 1-hour infusion) followed by CBDCA (1,000 mg/sqm in a 3-5-hour infusion in 1 day—Jet regimen—every 3-4 weeks), or, as an alternative, E (200 mg/sqm) and CBDCA (600 mg/sqm—Jet low-dose—in 1 day every 3-4 weeks). Since 1989, 129 children affected with solid tumors were treated with JET regimen and 10 of these (7.7%) suffered allergic reactions. Age at diagnosis of these 10 pts ranged from 8 mos to 23 yrs (mean: 8 yrs); 2 had neuroblastoma, 2 retinoblastoma, 2 medulloblastoma, 2 low-grade glioma, 1 high-grade glioma, and 1 osteosarcoma. There were 32 allergic reactions in these 10 children during 128 courses administered. The first reaction occurred after an average of 7 courses (after 1, 2, 2, 4, 6, 8, 8, 15, 21, and 22 courses). The severity of the reactions was grade 1 in 13, grade 2 in 13, grade 3 in 5, and grade 4 in 1, according to the toxicity criteria of the National Cancer Institute. No deaths occurred. Clinical symptoms were the result of a histamine-induced type I hypersensitivity reaction. In one case macroscopic hematuria was also observed after 2 hours.The first reaction occurred during or immediately after infusion with E after 2, 2, 4, and 15 courses, whereas it occurred during infusion or after 1, 6, 8, 8, 21, and 22 courses with CBDCA. The cumulative dose at which the first allergic reaction occurred ranged from 200 mg/sqm to 4,900 mg/sqm (average 1,610 mg/sqm) for E, and from 600 mg/sqm to 15,600 mg/sqm (average 6,100 mg/sqm) for CBDCA. In 1 pt chemotherapy was discontinued after the first grade 4 reaction, whereas 39 further courses were administered to the remaining 9 pts; in these, allergic reactions were observed in 22 courses (in 13 with Decadron-antihistamine premedication and in 9 without premedication), whereas no reaction occurred in 17 (5 with Decadron-antihistamine premedication and 12 without premedication). Allergic reactions can be unpredictable and in our series one grade 4 reaction after CBDCA infusion was observed during the first course, although hypersensitivity to CBDCA seems to be correlated with repeated exposure to the drug as reported in the literature. Furthermore, in E-CBDCA association, both drugs are potentially allergenic and it is sometimes difficult to attribute the allergic reaction to one or the other drug. Decadron-antihistamine medication may prevent the allergic reaction in some pts, although in others the allergic reaction can occur in spite of premedication.

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INTERFERON α-2A (ROFERON) IN THE TREATMENT OF HEMANGIOMAS OF INFANTS AND BABIES

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Hemangiomas are vascular tumors that occur in about 10% of the babies during the first year of life. While most hemangiomas are small and do not require treatment, other than cosmetic repair, others can obstruct vital organs and be life threatening ("alarming hemangiomas"). In our study we evaluated the effectiveness and safety of daily s.c. of interferon α-2a (IFN-2a) in untreated patients with skin and organ hemangiomas. From September 1992 to June 1995, 45 infants and children entered the study. Age at the onset of treatment ranged from 1 day to 96 months; the duration of treatment from 1to 9 months. To be eligible, patients were required to be previously untreated and to have either hemangiomas significantly distorting facial features or threatening vision or brain hemangiomas or abdominal hemangiomas. All patients received daily subcutaneous treatment of 1 x 10^6 U/m^2 IFN-2a the first week, followed by 2 x 10^6 U/m^2 the second week, and finally 3 x 10^6 U/m^2 daily afterwards. 28/45 patients were evaluable for response and all responded to treatment within 3 or more months of initiation of therapy. In 80% of patients tumor regression was ≥50%. One patient had response to treatment but, after stopping therapy for one month, an increase in size of the hemangioma was noted. This again regressed when treatment was reinstituted with 80% reduction in size within a month. Two patients with Kasabach-Merritt syndrome had their thrombocytopenia normalized within 2 months after initiation of IFN treatment. All responses were evaluated by ultrasonography or MRI and photography. There were no febrile episodes or other side effects from IFN-2a in any patients during the treatment except for a slight transitory increase of SGOT and SGPT up to five times of the baseline value. This non-randomized study demonstrate the safety, practicalness, and efficacy of interferon alfa-2a as primary therapy for infantile and childhood hemangiomas.

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EFFECT OF ETOPOSIDE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF METHOTREXATE IN VIVO

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The effect of etoposide on the pharmacokinetics of methotrexate was examined *in vivo*. High dose (5 g/m^2/24hr) methotrexate therapy was combined with two etoposide (100 mg/m^2/1hr) infusions as a part of the medulloblastoma protocol developed in our department. Vepesid therapy was administered in two different schedules. The first group of patients received etoposide immediately before and at the end (24th hr) of methotrexate treatment. The second group was treated with etoposide at 24 hrs and at 48 hours after starting methotrexate infusion. In this latter group both treatment related grade III and grade IV toxicity developed more frequently than in the first group (58.6 %, vs 29.2 %, for grade III toxicity $p = 0.019$, for grade IV toxic signs $p = 0.040$, respectively). We observed that after the second dose of etoposide given at 48 hr (second group) both total and unbound serum methotrexate levels (determined by high performance liquid chromatography) were elevated by 53.14-109.19 %, and 25.86-64.95 %, respectively by the third hour after completion of Vepesid infusion. This effect was detectable for 6 hours. All the liver and kidney functions of the patients were in the normal range. These results suggest the possibility of partial recirculation of extra/intracellular methotrexate into the blood after etoposide administration. Based on these results the therapeutic protocol has been modified and Vepesid is given prior to and at the end (24 hr) of high dose methotrexate treatment. Under these conditions only a slight decrease of methotrexate elimination has been detected between the 25-28th hr. These results emphasize the role of possible schedule dependent interactions of cytostatic drugs.

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HEMATOLOGICAL TOXICITY OF CYTOSINE ARABINOSIDE (ARA-C) ADMINISTERED INTRAVENOUSLY (iv) OR SUBCUTANEOUSLY (sc) IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL).

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Introduction: The bioavailability of Ara-C may change when administered iv or sc and thus in some protocols is given only iv and in others the dose is reduced by 20% when given sc. In this study hematological toxicity in children treated with Ara-C administered iv or sc at the same dosage has been investigated.
Patients and methods: In the BFM based AIEOP (Italian Association of Pediatric Hemato-Oncology) ALL 9102 protocol for intermediate risk ALL Ara-C is given at the dose of 75 mg/sqm daily x 4/week, x 4 weeks (total 16 doses) during phase Ib of the induction treatment, which includes also Cyclophosfamide, 6-MP and I.T. chemotherapy. Until February 1993 the drug has been given strictly iv. Starting March 1993 an amendment of the protocol allowed to administer Ara-C sc for patients convenience. In two consecutive groups of patients treated with Ara-C iv (50 patients, 1st group) or sc (50 patients, 2nd group) at the same doses the following aspects were investigated: duration of Phase Ib (expected:47 days), incidence of leukopenia (WBC <500/cmm), neutropenia (WBC<200/cmm) and trombocytopenia (<50000/cmm) episodes; incidence of RBC or platelets transfusions; incidence of FUO episodes and days of hospitalization. Data were analyzed by "Student t" test.
Results: Mean duration of phase Ib in patients treated with Ara-C iv or sc was respectively of 54 and 60 days ($p<0.001$). Leukopenia episodes occurred in 14% of patients in both groups; neutropenia episodes in 44% and 56% of iv and sc patients respectively ($p=0.4$). The average RBC and platelet transfusions per patient were 1.6 and 0.16 in the first group and 1.8 and 0.22 in the second group. FUO and aplasia episodes were observed in 10% and 24% of iv and sc patients respectively ($p=0.1$). Hospitalization days per patient were 0.7 and 1.3 in the 1st and 2nd groups respectively ($p=0.2$).
Comments: These results, although on limited numbers of patients, suggest that Ara-C given sc may cause a mildly increased hematological toxicity compared to iv route. The administration of Ara-C sc is however convenient both for patients quality of life and health care costs related to iv injections. More extensive investigations are needed to evaluate if a reduction of the dose of the drug when given sc is justified.

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HIGH DOSE CYCLOPHOSPHAMIDE IN RESISTANT PEDIATRIC TUMORS

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Few studies have evaluated the activity of cyclophosphamide used as a single agent however the optimal dose has not been stablished. We investigated the antitumor activity, drug toxicity and feasability of individual scalonated cyclophosphamide dose (140, 160, 180, 200 mg/kg), administered in two consecutive days followed by GM-CSF (10 µg/kg/day). We treated 20 children, mean age 10 (range 3-17) with relapsed medulloblastoma/PNET (2), neuroblastoma (4), rhabdomyosarcoma (2), S. Ewing (4), hepatoblastoma (1), renal carcinoma (1), stesioneuroblastoma (1), osteosarcoma (2) and Wilms tumor (3). All of them have previously received cyclophosphamide and/or ifosfamide. Hematological toxicity was severe, but tolerable and of short duration. The maximum tolerated dose (MTD) was 180 mg/kg/cycle (grau IV neutropenia and plaquetopenia, 90%). There were three deaths due to sepsis (dose 140 and 160 mg/kg), associated with neutropenia. Favorable response was 60% (5 CR, 2 PFR, 5 PR). **CONCLUSION**: cyclophosphamide and GM-CSF were well tolerated, the average escalonated dose per patient was 2,4 gr/m2/week. The MTD associated with the administration of GM-CSF was determined to be 180 mg/kg/cycle.

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PROTRACTED ORAL ETOPOSIDE (E) THERAPY: PRELIMINARY RESULTS OF A FEASIBILITY STUDY.

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BACKGROUND: Preclinical data and experience with adults suggest that etoposide (E) is a highly schedule-dependent drug, although the optimal dosages and schedules of administration have not yet been well evaluated.
OBJECTIVE: The feasibility and effectiveness of prolonged oral E in children with pretreated high-risk malignancies was investigated.
PATIENTS AND METHODS: Oral E (50 mg/m^2/day for 10 consecutive days with an interval of 7 days between every course) was administered to 6 patients with pretreated high-risk malignancies. Age at diagnosis ranged from 36 to 132 mos (mean 70.5). Three patients were affected with neuroblastoma (NB) at stage IV with bone metastases, 1 pt with relapsed medulloblastoma (MB), 1 with ependymoma (EP) at the third relapse, and 1 with unresectable Ewing sarcoma (ES) at a chest wall site. Since April 1995, a total of 74 courses (range: 9 to 19) were administered to 6 children (average courses per patient:12.3). Evaluation of response was performed every 2 months by CT or MRI. The pts with NB (3), with MB (1), and with EP (1) were pretreated with long-term JET regimen (carboplatin associated with E without BM or PBSC rescue) for an average of 18 courses. This regimen resulted in 2 CRs, 2 VGPRs and 1 SD. The pt with ES was previously treated with standard dosages of cyclophosphamide, adriamycin, vincristine, actinomycin D, and local radiotherapy; this approach resulted in SD. Oral E was started in the 2 pts with NB who achieved CR and in the remaining 4 pts with evaluable disease.
RESULTS: In the 4 pts with SD no progression of disease was observed after an average follow-up of 6 mos. CR was mantained after 8 and 9 mos in the 2 cases with NB. Acute toxicity was not observed: WBCs >2000/mm^3 Hb >9.5 g/dl, PLTs >150,000/mm^3); alopecia was mild.
CONCLUSION: In this series protracted oral E allowed for complete remission or stable disease to be mantained in very high risk patients. This schedule of oral E is feasible without toxicity. However, a longer follow-up is needed for a definite determination of activity.
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CARBOPLATIN-BASED CHEMOTHERAPY FOR REFRACTORY OR RELAPSE CHILDHOOD SOLID TUMORS

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Children with cancer who experience refractory disease or relapse have a uniformly poor prognosis. Carboplatin [CBDCA], an analogue of cisplatin, has found application in the cancers that afflict more than one half of the children with malignancy. It has been marketed in Turkey for a year. We gave CDBCA-based chemotherapy to 8 pts. Ages of patients ranged from 2 to 16 years [median10] with relapsed or resistant solid tumors. Two different combination were used: ICE consisted of ifosfamide [I](1.5 gr/m²) plus etoposide [E] (100 mg/m²) x 3 plus CDBCA (400-550 mg/m²) i.v. on day 3, CEV consisted of CDBCA (same dose ICE on day 1) plus E (same dose and schedule as in ICE) plus vincristine [V] (1.5 mg/ m²) i.v. on day 1. ,G-CSF was given in 14 courses for prophylaxis. Details of the patients and toxicity patterns (grade III/IV myelotoxicities) are given in the Table.

No Diagnosis	Status	1st-CT	CT	RT	Resp.	Total CT*	Follow-up
#1. NHL	Relaps	BFM-90	CEV	(+)	CR	CEV(3)/ICE(2)	NED (6 mo.)
#2. RMS (GU)	Relaps	AVAC	CEV	(+)	PR	CEV (10)*	AwD (9 mo.)
#3. RMS	Mts.lung	VAIA	CEV	(+)	PR	CEV (5)	DwD (4 mo.)
#4. RMS (GU)	Refrac.	EVAC	CEV	(-)	SD	CEV(6)/ICE(2)	AwD (8 mo.)
#5. Ewing	Mts.lung	VADRC	CEV	(+)	SD	CEV (7)	DwD (7 mo.)
#6. Ewing	Refrac.	EVAIA	ICE	(-)	SD	ICE (2)	AwD (2 mo.)
#7. Ewing	Refrac.	EVAIA	ICE	(-)	SD	ICE (2)	AwD (2 mo.)
#8. Pons tm.	Refrac.	-	ICE	(-)	SD	ICE (3)	AwD (4 mo.)

Regimen	Growth fac.	No.of courses	Thrombocytopenia	Neutropeni	Septicemia
CEV	G-CSF (+)	7	3	3	0
	G-CSF (-)	25	0	1	0
ICE	G-CSF (+)	7	6	5	2
	G-CSF (-)	4	0	2	0

Number of courses

Carboplatin merits further investigation because of its potential advantages for children with cancer.

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SIGNIFICANCE OF THE IN VITRO DRUG SENSITIVITY ASSAY USING ALLOGENEIC BONE MARROW STROMAL CELLS FOR NATURAL KILLER (NK) CELL LEUKEMIA

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NK cell leukemia is a rare disorder and its prognosis is usually very poor. It is known that allogeneic bone marrow stromal cells support the growth of acute lymphoblastic leukemia cells. We applied this system to test the drug sensitivity of the NK cells from a 14 year old patient with NK cell leukemia. This patient had a huge splenomegaly in March in 1994. After splenectomy, her peripheral leukocytes increased to the maximum of 100,000 per micro liter. Most of these cells were large granular lymphocytes and they were $CD2^+$, $CD3^-$, $CD16^+$, $CD56^+$, $CD57^-$ with a high NK activity. T-cell receptor genes were not rearranged and the only two bands of EB virus genome were detected by Southern blotting. The X-chromosome inactivation assay revealed that these cells were monoclonal and NK cell leukemia was diagnosed. We used a bone marrow stromal layer established from the Dexter type long term culture of allogeneic bone marrow cells. NK leukemic cells were incubated on this layer for 7 days with various kinds of anti-leukemic agents. NK cells were very resistant to Ara-C and Mitoxantrone and they were sensitive to Steroids. 2-chloro-deoxyadenosine showed a very modest cytotoxicity. Notably, both IL-2 and IL-4 stimulated the proliferation of NK leukemic cells. These results were similar to the patient's clinical response to the drugs. We stopped using the ineffective drugs and initiated Steroids and Cyclophosphamide-based treatment and the patient's leukemia was induced into remission. She has been in continuous remission for 15 months now (February in 1996) while she is still receiving monthly cyclophosphamide. The drug sensitivity assay may be useful for the leukemia for which a standard therapy is not established.

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PHARMACODYNAMICS AND DRUG SYNERGISM STUDIES OF PEG-ASPARAGINASE (PEG-ASNase) AND CYTARABINE (ara-C) IN LEUKEMIA CELL LINES (CEM/0 and CEM/ara-C-7A).

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Repetitive treatment of pediatric patients with E. Coli asparaginase (ASNase) induces hypersensitivity reactions to this drug in as many as 66% of patients. We have developed the rabbit anti-asparaginase antibody and the following assays: 1) the ELISA assay for PEG-ASNase in serum; 2) the serum antibody titers for either E. coli or PEG-ASNase; 3) the enzymatic assay for ASNase specific activity through the nesslerization of NH_3 and the oxidation of NADH to NAD^+; and 4) the HPLC assay of the derivatized (PITC) amino acids, specifically, asparagine, aspartic acid and glutamine. We have performed combination studies with these drugs in the leukemia cell lines (CEM/0) and CEM/ara-C-7A, which lacks 50% of the mRNA for deoxycytidine kinase signal. The IC_{50} values of ara-C were 0.032 μM and 0.11 μM in these lines, respectively. The IC_{50} values of PEG-ASNase alone were 0.002 IU/ml and 1.52 IU/ml, respectively. The ara-C resistant cell line CEM/ara-C-7A is also 681-fold cross-resistant to PEG-ASNase as compared to CEM/0. The concurrent drug administration for 48 hours resulted in IC_{50} values of 0.56 nM for ara-C and 0.56 mIU/ml for PEG-ASNase in CEM/0 or a 57.4-fold synergism compared to ara-C alone, as estimated by the Median Effect Principle theory. In the CEM/ara-C-7A cell line, the co-incubation for 48 hours resulted in IC_{50} values of 15 nM for ara-C and 15 mIU/ml for PEG-ASNase or a 7.25-fold synergism as compared to ara-C and 101.1-fold synergism in comparison with PEG-ASNase alone. The combination of ara-C and PEG-ASNase in this cell line produced significant collateral sensitivity to PEG-ASNase. We determined that these drugs are highly synergistic against leukemia cell lines sensitive or partially resistant to ara-C. Hence, we conclude that the use of PEG-ASNase in combination with nucleoside anabolites may benefit leukemia patients with early relapse. In vitro studies with PEG-ASNase and rabbit anti-ASNase antibody developed against E. coli ASNase showed that a strong cross-reactivity (80%) exists. Since this interaction may be occuring in patients' plasma, the implications of this is significant and may affect clinical outcome. Clinical pharmacodynamic studies are being performed in pediatric patients with ALL to answer these questions.

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COMPARISON OF ORAL VS. INTRAVENOUS ETOPOSIDE: AUC-DISTRIBUTION AS RATIONALE FOR ORAL TREATMENT

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Introduction: Orally applicated etoposide is considered to be less reliable than its iv. administered form. The anti-tumour activity against various tumor types has been associated with the maintenance of low etoposide levels (0.5-3μg/ml), whereas levels > 3 μg/ml correlate strongly with neutropenia. (Clark, SCLC, JCO'94, Hainsworth, various Tu., 'Cancer Chemother Pharmacol. '94).Thus, the therapeutic index seems to be best within limits of 0.5-3 μg/ml. In this context it is interesting to compare the AUC -variability following iv. and oral application within the limit of 0.5-3 μg/ml, in addition to compare the respective AUC∞ as a whole.

Patients and Methods: 11 patients received etoposide (42-149 mg/m², median 88 mg/m²) orally, 17 children received etoposide as short-time infusion (67-200 mg/m², median 150 mg/m²) within different treatment schedules. A mean of 8.5 (oral) and 6.8 (iv.) samples were taken per 24h. Etoposide was measured using HPLC. To ensure comparability, we normalised the data to 100 mg/m².

Results:

Etoposide	iv (100mg/m²)	oral (100mg/m²)	p =
• AUC-Values / Exposure [h] to etoposide serum-ranges			
AUC∞ total[μg*h/ml]	83,2	45,7	0.0003
% of AUC∞ [μg*h/ml](>3μg/ml)	44,2 %	14,1 %	< 0.0001
% of AUC∞ [μg*h/ml] (0.5-3μg/ml)	43,1%	50 %	0.027
% of AUC∞[μg*h/ml] (< 0.5μg/ml)	14,4 %	36,1 %	< 0.0001
Exposure [h] to >3μg/ml	9,7	4,2	0.0004
Exposure [h] to (0.5-3μg/ml)	9	13,8	0.0024
Exposure [h] to (< 0.5μg/ml)	5.53	4,8	0.52
• Coefficients of Variability			
AUC∞ total[μg*h/ml]	30 %	41,1 %	
AUC > 3 [μg*h/ml]	18,7 %	100,7 %	
AUC 0.5-3[μg*h/ml]	14,1%	13,7 %	
AUC< 0.5[μg*h/ml]	16,7 %	33,1 %	

Conclusion:

The difference in total AUC∞ following oral or iv administration is primarily due to differences in etoposide levels below 0.5 and above 3 μg/ml. Based on the assumption of Clark and Hainsworth a rational therapeutic aim seems to be the achievemt of serum levels of 0.5-3μg/ml. After oral drug intake, the AUC within these limits amounts to 80% of the corresponding iv. values. As the interpatient- variability is comparable between the two groups within these etoposide levels, the oral form of etoposide is just as reliable as its respective iv form in intermediately dosed etoposide schedules.

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HIGH RATES OF CHILDHOOD CANCER IN MUSLIM ASIANS FROM THE WEST MIDLANDS, UK. IS PARENTAL CONSANGUINITY RESPONSIBLE?

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Nearly 10% of the 1 million children who live in the West Midlands Health Authority Region of the UK are of Asian (Indian, Pakistani and Bangladeshi) ethnic origin. These Asian children have previously been shown to have a higher incidence of cancer than their white counterparts.

Consanguineous marriages are very common in the Muslim community, but rare among the non-Muslim Asians. To investigate whether consanguinity increases the risk of childhood cancer, we derived cancer incidence rates for Muslim and non-Muslim Asian children. Congenital abnormalities in these cancer patients, and in their close relatives, were also recorded.

Between 1978 and 1992, 187 Asian children with cancer were registered. Of these, 54% were Muslims, mainly from Pakistan and Bangladesh. Cancer incidence was significantly higher in Pakistani / Bangladeshi Asians, with an age-standardised incidence of 163 cases per million per year, compared to 115 for Indians (non-Muslims) and 125 for Whites.

Among Asian cancer patients, congenital malformations were significantly more common in the Muslims (21%) than in the non-Muslims (7%, $p<0.01$). Eight percent of the Muslims suffered from autosomal recessive conditions and 5% had autosomal dominant disorders. The proportion with sporadic genetic conditions was similar in both groups.

Of the Muslims with malformations, 48% had a cancer malformation syndrome, and in 33% of cases there was a close relative with both a childhood cancer and a malformation.

Genetic factors are predominantly responsible for the cancer excess in Muslims. Consanguinity contributes to the prevalence of cancers related to autosomal recessive disorders. However, many cancers are linked to autosomal dominant disorders and these may be a consequence of older paternal age at conception, in the current or previous generations.

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EARLY AND SEVERE LUNG FIBROSIS IN CHILDREN TREATED FOR MALIGNANT GLIOMAS WITH THE BCNU, CISPLATIN AND VP 16 (BCV) REGIMEN.

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Introduction. Lung toxicity of BCNU, one of the most active drugs in the treatment of malignant brain tumors, is known as a late and dose-dependent complication.

Patients and methods. From March 1990 to January 1995, 85 patients (pts), 3-19 years of age (median : 10), harboring a high grade glioma have entered a pilot study of chemotherapy combining BCNU (40 mg/m^2/d x 3 over 1 hour), cisplatin (30 mg/m^2/d x 3 over 3 hours), VP16 (150 mg/m^2/d x 3 over 1 hour). No pts had previously received nitrosoureas and/or spinal radiotherapy. For the 66 newly-diagnosed pts, the BCV combination was discontinued until no further improvement were observed on CT scan or MRI, performed after each new 2 courses, which was then substituted by radiotherapy (RT). Because 2 cases of interstitial pneumonitises occurred in the first pts, the dose of BCNU was decreased by 50% in the fifth and sixth course. One to 7 courses (median : 4) were delivered. The median cumulative dose of BCNU was 480 mg/m^2 (range 120 to 840). The median interval between courses was 27 days. Fifty-six percent of the patients are NED with a median follow up (FU) of 38 months (3-55 m).

Results. Eight interstitial pneumonitises occurred after 4 (3), 5 (2), and 6 (3) courses, 3 to 9 months after the first course of BCV. That represents 18% of pts (43) who received at least 4 courses and with a FU > 6 months. Bronchoalveolar and microbiological assessments were always negative. Ages (3 to 15, m : 9 y) were not different from the whole group. Pneumonitises occurred during or soon after a HDCT (thiotepa + VP16) + ABMT regimen in 3 cases, which led to 2 deaths. Among the other 5 pts, 3 were cured with steroids (12m$^+$, 38m$^+$), 1 still requires steroids (52m$^+$) and 1 died of pneumonitis.

Discussion. A high incidence of fatal pulmonary fibrosis has previously been described with an overall mortality of 47% occurring from 1 to 12 years post treatment (O'Driscoll et al. Late carmustine lung fibrosis. Chest 1995, 107, 1355-7). This toxicity seemed to be age-dependent (major risk for children < 6 y) and gradual with time but dose independent. Our series is particular because pneumitises occurred very early (median = 4), with moderate cumulative doses, and seems to be enhanced by a HDCT regimen. The role of dose-intensity could be incriminated (120 mg/m^2/4 weeks versus 100 mg/m^2/6-8 w in O'Driscoll series). A prospective longitudinal study of pulmonary function is running. BCNU at these dose and schedule, should be avoided in children.

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RESTRICTION OF THE MYOCARD; A LATE SIDE-EFFECT OF TREATMENT WITH ANTHRACYCLINES

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Development of myocardial dysfunction, during and after treatment for childhood malignancy with anthracyclines, is well documented. Serial echocardiographic monitoring during treatment is mandatory but the question may be raised over which period of time to continue cardial evaluation after cessation of therapy. We assessed the incidence of late asymptomatic cardiac dysfunction in patients with normal echocardiographic findings at completion of anthracycline treatment.

A total of 97 patients (pts) (48 girls and 49 boys) treated for childhood malignancy, who had completed anthracycline therapy 5.5 to 18.6 years before, were restudied by echo-Doppler cardiography. A left ventricular shortening fraction (LVSF) of $\leq$ 25% was interpreted as abnormal.

Results: Twelve pts (12.3%) had a LVSF of $\leq$ 25%. Total dose anthracyclines received by these pts was 160 - 850 mg/m^2. Left ventricular end-systolic dimension had increased in 14.4% of the pts (inclusive 12 pts with abnormal LVSF). Left ventricular end-diastolic dimension had decreased in 13.4% of the pts.

Left ventricular posterior wall thickness was below P5 in 40.2% of the pts. The interventricular septum thickness was below P5 in 58.7%. The E/A ratio of the mitral valve diastolic flow pattern was within normal range.

Conclusions:

1. Congestive cardiomyopathy was demonstrated in 12.3% of the pts.
2. In a higher percentage of the pts a decrease of the ventricular wall- and septum thicknesses was demonstrated without dilatation of the left ventricle which could comply with a form of restrictive cardiomyopathy.
3. Monitoring of the heart function seems to be mandatory in pts long after cessation of anthracycline therapy.

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DEFECT IN BONE MINERALIZATION FOLLOWING IFOSFAMIDE (IF) TREATMENT FOR CHILDHOOD SARCOMAS.

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Several therapeutic trials of IF have documented its efficacy in various pediatric sarcomas. However concerns have been raised about the renal tubular dysfunction leading in some patients to rickets associated with the administ ration of IF. Whether the decreased mineralization of bone induced by IF is permanent or not is unknown, mainly due to the limitations of current bone measure ment techniques. The recent adaptation of quantitative computed tomography (QCT) to measure the mineral density of cortical bone in the peripheral skeleton has significantly improved our ability to quantify bone mineralization in vivo. This study was undertaken to investigate the degree of mineralization of bone in ten children with bone and soft tissue tumors who were treated with IF. All children were in complete remission at the time of the study and had been off theapy for at least four monts. They had received a mean IF total dose of 60 G/M^2 (range, 18-117). CT values for bone mineralization in these patients were compared to a group of healthy children matched for race, sex, age, height and weight. Compared to controls, patients treated with IF had substantially less mineral in bone ($p < 0.05$); on average treated patients had a 10% reduction in the amount of mineral per unit volume of bone. The decrease in bone mineralization was independent of the time since completion of therapy ($r = -0.06$) and persisted in patients that had been off therapy for more than three years. These results suggest a defect in mineralization, associated with IF which may be longlasting. Prospective longitudinal studies are needed to determine whether this abnormality is reversible.

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DOSE-RELATED NEPHROTOXICITY OF CARBOPLATIN

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The aims of this study were to document renal function after treatment, and to investigate risk factors for the development of chronic renal damage in children receiving carboplatin. 23 newly diagnosed patients with cancer (10F; mean age 4.6 years, range 0.4 - 15.4), who received carboplatin but not cisplatin or ifosfamide were studied. After treatment all patients underwent assessment of glomerular, proximal and distal renal tubular function. The mean cumulative dose (CD) of carboplatin was 3.4 g/m^2 (range 1.4 - 7.1). The main abnormalities were in the serum magnesium (SMg) and the glomerular filtration rate (GFR). The mean SMg (range) at 1 and ≥6 months was 0.71 (0.42 - 0.87) and 0.76 (0.59 - 0.9) mmol/l [normal range 0.7-1.0]. One patient (CD carboplatin 6 g/m^2) suffered a fit associated with hypomagnesaemia and remains dependent on supplements. The mean GFR (range) at 1 and ≥6 months was 112 (69-175) and 107 (63 - 170) ml/min/1.73 m^2. The mean fall in GFR from before treatment started to 1 month after it finished was 27 ml /min/1.73 m^2 (95%CI 5-48), and from before to ≥ 6 months after treatment was 30 ml/min / 1.73 m^2 (95%CI 10-49). There was a negative correlation between CD of carboplatin and S Mg at ≥ 6 months post treatment (p=0.044). No clinically important renal damage was seen with carboplatin CD ≤3000 mg/m^2. High CDs of carboplatin can be used with safety, however may be associated with evidence of renal damage qualitatively similar to that caused by cisplatin but with much less severity. GFR and SMg should be carefully monitored when high CDs of carboplatin (>3 g/m^2) are used, and if it is used with other nephrotoxic chemotherapy such as cisplatin or ifosfamide.

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OSTEOPENIA AND GROWTH HORMONE(GH) INSUFFICIENCY IN ADULTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) IN CHILDHOOD.

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Therapy directed at the central nervous system in childhood ALL is important in maintaining long term survival, and until recently has been achieved with pre-symptomatic cranial irradiation (XRT). GH deficiency is a well recognised sequela of this therapy and is related to the dose of XRT, the fractionation schedule and the post irradiation time interval. GH therapy in GH deficient adults is now a possibility with clear benefits to bone mineral density (BMD), quality of life, body composition and exercise capacity, but the GH status in adult survivors of childhood ALL has yet to be studied extensively.

We, therefore, have assessed the GH status and the bone mineral density in 22 [median age 23.3 (20-33) years, 13 male] adult survivors of childhood ALL who received between 18 - 25 Gy XRT [median age at XRT 8.3 (1.7 - 16) years]. No subject had received GH or other hormone replacement therapy. GH status was measured by provocative testing with an insulin tolerance test (ITT) and arginine stimulation test (AST). BMD was measured using dual energy x-ray absorptiometry (DEXA) in the lumbar spine and the femoral neck, and by single photon absorptiometry (SPA) in the forearm. Five subjects "failed" (peak < 9mIU/L) both GH tests indicating severe GH deficiency, all had received 24-25 Gy XRT. An additional 9 subjects had a peak GH response between 9 - 20 mIU/L [3 received 18 Gy, 6 received 24-25 Gy].There was a significant reduction in BMD at the lumbar spine (median Z score -0.82, p = 0.004), and at the forearm (median Z score -1.5 p = 0.001) but not in the femoral neck (median Z score - 0.23, p = 0.6). We conclude that a significant number of adults treated for childhood ALL are GH insufficient and have a reduction in bone mass.

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LONG TERM EFFECT OF INTERFERON (IFN) TREATMENT OF CHRONIC HEPATITIS B (CHB) IN PEDIATRIC PATIENTS CURED OF ONCOLOGIC DISEASE.

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Pediatric patients, who acquired HBV infection during a neoplastic disease may show tolerance to the virus. Long term effect of IFN treatment in these patients is not yet established. We treated 8 patients (aged 8 to 25 years, M/F 4/4), with a CHB of mild (5 pts) to moderate (3 pts) activity, who were off therapy for a previous leukemia or solid tumor from at least 2 years. The patients received α 2a IFN, 5 MU/mq t.i.w. for 6 to 12 months.During treatment 5 pts who had a basal HBVDNA levels < 100 pg/ml cleared HBVDNA and seroconverted to HBeAb (Complete response). Two pts with baseline serum HBVDNA > 100 pg/ml did not respond to treatment. One pt cleared HBVDNA, but showed no seroconversion to HBeAb (Partial responder). All pts have been followed-up for 6 to 24 months after discontinuation of therapy. Non responder patients as well as the partial responder still have active viral replication. Of the 5 responder pts, 2 showed a sustained response at 6 and 18 months, respectively; 2 showed a transient reactivation with low levels of serum HBVDNA and reappearance HBeAg, followed by a spontaneus remission few months later (HBeAb positive). The other patient showed a relapse after both the first and the second course of IFN, conducted for 6 and 8 months respectively. He is now receiving a third IFN course and is again in complete remission. The data show that chronic HBV infection, acquired during a neoplastic disease, may respond to IFN treatment, but tend to recur. In some pts, additional IFN courses may be necessary to maintain a complete response. (Supported by MURST 40%, Italy)

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THYROID FUNCTION IS NOT AFFECTED BY SECOND EXPOSURE TO ERWINIA ASPARAGINASE (ASP) FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Impaired thyroid function has been associated with first exposure to ASP given for ALL treatment. We investigated the effect of ASP administration on thyroid function in intermediate risk ALL children during second exposure to ASP as part of intensive, BFM-based, AIEOP ALL-9102 chemotherapy. Pts were randomized to receive high dose (HD 25,000 IU/m^2/week x 20) ASP from the beginning of reinduction therapy, vs standard dose (10,000 IU/m^2/3-4 days x 4). Twenty-six consecutive pts (20 males) were studied. Nine had been randomized to receive HD-ASP and the other 17 standard-dose ASP. Blood samples were collected on weeks 0, 1, 4, 9, 12, 30 from the beginning of reinduction therapy and stored until used. Plasma levels of T3, free T3 (fT3), reverse T3 (rT3), T4, fT4, TSH, TG, TBG were determined on any sample. In 13 pts (6 at HD) TRH test was performed on weeks 0 and 9.

No patient had clinical nor biochemical signs of hypothyroidism. There was no significant difference in thyroid hormone values between the two patients groups. In all patients on week 1 T3 and fT3 levels were within the normal range but significantly reduced ($p<.005$) compared to week 0, with a contemporary transient increase of rT3 values ($p<.005$); reversal of this pattern was complete. T4 and fT4 values were stable. TSH mean values were normal even on week 1, during transient reduction of T3 and fT3. TG values were steadily low-normal. TSH values after TRH stimulation had a normal pattern (peak time and height, AUC) at both evaluations.

Intensive BFM-based reinduction chemotherapy for childhood ALL is associated with mild, transient reduction in T3 and fT3 values. This is consistent with definition of "low T3 syndrome" and might be related to high dose dexamethasone therapy. Second exposure to short, standard-dose ASP or to extended HD-ASP did not affect thyroid function.

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"AFTER CURE" - AN INFORMATION BOOKLET FOR ADULT SURVIVORS OF CANCER IN CHILDHOOD

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With the advances in treatment for childhood and adolescent cancer there has been a significant increase in the number of survivors entering adulthood. All survivors should be followed to monitor the incidence and evolution of sequelae of treatment as it is known that some of this population face important late effects. For some survivors the risk of late effects may be predicted at the time of treatment but for many the type and extent of such risks are uncertain.

Education of the survivors is likely to be one of the most effective ways of ensuring surveillance and influencing health related behaviour. This is also an effective means of informing future medical carers of the problems these individuals may encounter.

The booklet "After Cure" is written for survivors to enhance discussions with staff at longterm follow up clinics. The contents are in two sections, the first includes general information and the second section provides specific information dealing with issues applicable to the individual concerned. Some of the topics in the first section include the rationale for longterm follow up, information about employment and general advice about a healthy lifestyle. Material for the second section includes topics such as fertility, risks of cardiotoxicity and breast screening.

The value of the booklet is being assessed by a pilot study looking at a population of patients attending adult longterm follow up clinics from which data will be presented. This evaluation will influence the final version of the booklet which, is intended for wide distribution within the UKCCSG.

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DISTURBANCES IN ORAL AND DENTAL STRUCTURES IN PEDIATRIC LONG-TERM SURVIVORS OF LYMPHOMA FOLLOWING CHEMOTHERAPY: A Preliminary Report

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As the prognosis of chidhood cancer has improved, more attention has been focused on the long term side-effects of various treatment modalities. Cancer therapy may cause profound systemic, craniofacial and dental abnormalities.

The aim of the present retrospective study was to evaluate these abnormalities in the lymphoma group comprising about 13% of childhood malignancies. Decayed teeth, tooth eruption, missing teeth, discoloration, craniofacial growth, periodontal status and other abnormalities that can be observed in the oral cavity were detected.

To date 20 children with lymphoma in remission were evaluated 1/12-5 years after cessation of the chemotherapy. Ages ranged between 4-15 years (median 9 5/12 years). 17 of the patients were boys. All of the patients had received chemotherapy and four patients had additional radiotherapy. 20 age, sex and socioeconomic status matched healty children served as controls. Oral/dental and radiographic examinations were performed in both groups.

The results revealed that, most of the children who had received chemotherapy had displayed root malformations, enamel hypoplasia and opacities, missing teeth and delayed tooth eruption. Tooth discoloration / opacities and agenesis were found significantly higher in the study group($p < 0.05$). The craniofacial growth seemed not to affected in 5 to 7 years age group. Results of this study were evaluated in comparision with previous studies and relevant literature.

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SCHOOL BEHAVIOUR AND HEALTH STATUS AFTER CENTRAL NERVOUS SYSTEM TUMOURS

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Introduction
Brain injury due to the primary pathology, in addition to CNS targeted therapy, is likely to potentiate problems of re-integration into society for both the patient and their families following treatment of CNS tumours. The nature and extent of these difficulties is not comprehensively documented.
Method
Established questionnaires directed at measuring school behaviour(Deasy-Spinetta), play performance (Lansky) and health status(modified McMaster Health Utility Index) were completed by teachers for 27 children following treatment for CNS tumours, 25 of their siblings and age- and sex-matched controls. Patients aged over 9 years and all parents were asked to complete a Health Utility Index questionnaire.
Results
Patients demonstrated a reluctance to participate in organised physical activities($p=0.03$), worried more than matched controls($p=0.04$) and had reduced overall health status($p=0.049$). Siblings scored normally although were less likely to show concern for others($p=0.02$) or openly express joy($p=0.003$). Neither radiotherapy nor age at diagnosis had a significant effect.
Conclusions
These results confirm the widely held view that survivors of CNS tumours experience significant behavioural problems. This is in contrast to similar studies in tumours outside the CNS. They must be highlighted as a special needs group in order to enhance their social reintegration and health-related quality of life.

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LONG-TERM ADVERSE EFFECTS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH CANCER

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Transplantation of hematopoietic stem cells is a therapy of choice for some children with high- risk malignancies. As it is combined with highly toxic chemotherapy and/or irradiation its use is associated with both early and late morbidity and mortality. Long-term follow up of all patients is therefore essential in our continuing efforts to improve this therapeutic modality. In our institution we have transplanted a total of 33 children (21 boys and 12 girls, mean age 12.6 years, range 1.3-20.3 y) in 1992-1994. The average follow-up period of the patients (including pre-transplant) is currently 67.7 months (13.1-126.9, median=72). The group is subdivided also according the diagnosis and the pre-transplantation therapeutic protocol used. In 30 pts autologous grafts (either BM or PBSC) were used; 3 pts received allogeneic sibling grafts. Eighteen pts (54.5%) are alive; 14 (42.4%) in CR, 1 (3%) in PR and 3 (9.1%) with disease progression. Fifteen pts died; 3 in the peritransplant period [(30d)-2 of mycotic sepsis and 1 of multiple organ dysfunction]. No mortality occured in the early post -transplant period (30-100d). Symptoms of organ toxicity were recorded as per the CCG 3891 protocol; the following side-effects were noted according the WHO criteria: Hepatopathias 1st degree in 2 pts (6.7%), 2nd degree in 1 pts (3.3%); nephropathias 1st degree in 3 pts (10%), 2nd degree in 1 pts (3.3%); pneumopathias 1st degree in 4 pts (13.3%), 2nd degree in 2 pts (6.7%), 3rd degree in 3 pts (10%); cardiomyopathias in 1 pt requiring medication; slightly compromised hearing in 4 pts (13.3%), partial hearing loss requiring aid in 1 pt (3.3%); sicca syndr. in 1 pt (no eye cataracts were observed). Growth retardation (>2SD) was observed in 2 pts (6.7%); amenorrhea occured in 7 girls (70%). So far the limited number of pts in the follow-up does not allow for statistical correlations with the diagnosis and the protocols used; a higher incidence of pneumopathias and growth retardation seems to occur in patients with TBI. The above outcomes seem to be in line with reports from other paediatric BMT centres.

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HIGH PREVALENCE OF LEARNING PROBLEMS IN CHILDREN TREATED FOR LEUKEMIA AND LYMPHOMA IS EXCLUSIVELY RELATED TO CRANIAL IRRADIATION: A COMPARATIVE STUDY

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Aim: Prophylactic treatment of the central nervous system (CNS) in childhood leukemia and lymphoma is associated with negative effects on intelligence. We investigated the clinical significance of these findings by comparing the effect of different types of CNS prophylaxis on the survivor's learning capabilities.
Method: To isolate the effect of different types of CNS prophylaxis from other treatment and disease variables on learning problems, children treated for leukemia or lymphoma who received CNS prophylaxis with cranial irradiation (n=30) or without cranial irradiation (n=36) were compared with children treated for solid tumors who received systemic chemotherapy without any CNS treatment (n=30) and with healthy matched controls (n=265). Learning problems were assessed by standardized parent as well as teacher report.
Results: Relative frequencies of learning problems are shown in the table.

	Childhood cancer survivors (*N*=96)			Matched control group (*N*=265)
	With CNS prophylaxis		Without CNS prophylaxis (*n*=30)	
	Cranial irradiation (*n*=30)	No cranial irradiation (*n*=36)		
Serious learning problems	40% *(n=12)*	8% *(n=3)*	10% *(n=3)*	4% *(n=11)*
Mild learning problems	40% *(n=12)*	6% *(n=2)*	10% *(n=3)*	13% *(n=35)*
Total learning problems	80% *(n=24)*	14% *(n=5)*	20% *(n=6)*	17% *(n=46)*

Children who received cranial irradiation had significantly more learning problems ($p < 0.0000$) than children treated with intrathecal chemotherapy only. Similar low frequencies were found in childhood cancer survivors without any form of CNS prophylaxis and in healthy matched controls.
Conclusion: The high prevalence of learning problems in survivors of childhood leukemia and lymphoma is exclusively related to cranial irradiation.

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LUNG FUNCTION IN SURVIVORS OF CHILDHOOD LEUKAEMIA

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An earlier study of survivors of childhood leukaemia demonstrated a significant reduction in lung volumes and fall in transfer factor when compared with age and sex matched controls. A follow up study of the same group of patients was performed to reassess their lung function.
Of the original 70 subjects, 51 attended for the second study. All patients performed full spirometry. Transfer factor (DLCO) and transfer coefficient (KCO) were measured in 48. A further 20 control subjects were added to the original cohort of matched controls to allow for the increase in age of the survivors. Regression equations were re-calculated for each index of lung function using the control data. Results were expressed as a percentage of the predicted value for each individual (for the given age, sex and height) for each index of lung function. The mean of these values for each group were compared for visits 1 and 2 using a 2-tailed t test and are presented with 95% confidence limits.
The mean age of patients at study 1 was 14.36 (12.9-15.9) years and at study 2 was 17.53 (15.9-19.1) years. The mean age of the control group (n= 168) was 16.07 (15.09-17.05) years.
Forced expiratory volume in one second [study 1]= 89.4% (85.1-93.7), [study 2]= 84.7% (80.2-89.1), $p<0.05$. Forced vital capacity [study 1]= 91.2% (87.6-94.8), [study 2]= 85.9% (81.9-90.0), $p<0.05$. Total lung capacity [study 1]= 92.6% (88.4-96.9), [study 2]= 84.3% (81.2-87.4), $p<0.05$. Residual volume [study 1]= 101.8%, [study 2]= 85.0% (76.7-93.3), $p<0.05$. There was no significant fall in DLCO [study 1]= 91.6% (86.6-96.6), [study 2]= 92.2% (87.4-97.0).
The results demonstrate a further fall in lung volumes in survivors of leukaemia. One possible explanation is a relative lack of lung growth through puberty.
Further follow up of lung function in survivors of childhood leukaemia is indicated.

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SATISFACTION AFTER LIMB SPARING (LS) AND AMPUTATION (AMP) FOR MALIGNANT BONE TUMORS.

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We surveyed patients who had undergone LS surgery or AMP for a malignant bone tumor to assess their satisfaction with the functional outcome of the procedure and its impact on their quality of life. Eligible patients were ≥ 13 years of age and in remission ≥ 1 year after surgery. Thus far, 47 of 130 patients have responded to the questionnaire (45 treated for osteosarcoma and 2 for Ewing's sarcoma). Surgery comprised AMP in 38 (81%) cases (23 above knee, 7 hip disarticulation, 5 below knee, 1 hemipelvectomy, 1 above elbow, 1 forequarter), LS in 7, and LS followed by AMP in 2. The median age at surgery was 15 years (range, 3.3-23.11) and the median time since surgery was 12 years (range, 1.8-30.4); 29 patients (62%) were female. Currently 13 (28%) of respondents are students and 25 (53%) are employed full-time. Of the 31 patients who work outside the home 13 (42%) reported that AMP/ LS had influenced their choice of employment. However, the majority reported that AMP/LS had NOT caused a job refusal/ change (81%) or job loss (90%); affected salary (90%), chances for raise or promotion (90%), working relationship with employer (87%)/ coworkers (100%), or work performance (87%)/ responsibilities (97%); or necessitated modifications of the workplace (90%). Functionally, 42 (89%) felt they could perform most tasks; 25 (53%) could do most tasks without difficulty. Fifteen patients (32%) were extremely satisfied, 19 (40%) very satisfied, and 13 (28%) somewhat satisfied with their functional abilities. Surprisingly, 27 (57%) reported pain in the preceding month. A comparison of the impact of AMP vs LS on educational and occupational achievement, functional limitations, pain, emotional status, interpersonal and social interactions, rehabilitation and general life satisfaction in a larger sample of respondents in this ongoing study will be presented, along with implications for clinical management and rehabilitation.

P-250

CARDIOPROTECTION IN CHILDREN TREATED FOR LEUKAEMIAS WITH ANTHRACYCLINES

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Introduction of anthracyclines into chemotherapy protocols for the treatment of leukaemias resulted in remarkable progress in the treatment outcome. The huge benefit however, has to be balanced against the risks of early and delayed cardiotoxicity.
Different approaches to circumvent this untoward effect of antyhracyclines have been reported in literature.
Recently, cardioprotective efficacy of ICRF-187, Cardioxane (CDX) has been documented in adults and children.
Until today , in our clinic, 37 acute leukaemia patients (age 1-16 years) were treated according to the indicated BFM chemotherapy protocol and concomitant CDX.
The cardioprotective agent was administered in short term infusion, 30 minutes prior to anthracycline. The ratio between CDX and anthracycline was 10:1 or 20:1. The total anthracycline dose ranged between 150-360 mg/m^2.
The cardiac performances (LVIDd, LVIDs, LVEF, FS, E/A, IVRT and acceleration and deceleration of LV inflow) were assessed at the beginning of the chemotherapy, before consolidation phase and 1 and 6 months after anthracycline administration.
Presently, we are reporting the data from 20 patients that have completed the examinations.

Systolic and Diastolic Echo-Doppler Left Ventricle Performances

	before anthracycline administration	6 months after	p
SF (%)	36±7	34±6	NS
LVEF (%)	65±8	63±7	NS
E/A	1,6±0,4	1,5±1,4	NS
Acc (m/s^2)	7,1±1,9	7,2±2,3	NS
Decc (m/s^2)	8,5±3,1	8,0±2,6	NS
IVRT (ms)	49±16	52±19	= 0,03

Student's t-test

Until now, in our series of patients, we did not observe any significant impairment of LV systolic or diastolic functions. However, the final assessment of the cardioprotective efficacy of CDX will be possible only after long term follow up of the patients treated.

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LONG TERM CARDIAC EFFECTS OF ANTHRACYCLINES THERAPY: AN ECO-DOPPLER STUDY OF LEFT VENTRICULAR FUNCTION IN PATIENTS TREATED WITH ADRIAMYCIN FOR EWING'S SARCOMA IN CHILDHOOD.

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Patients-From may to novembor 1995 9/45 patients (pts.) off therapy, treated for Ewing's Sarcoma at 3rd Departement of Pediatrics-University of Bologna, diagnosed from july 1977 to january 1990, have been evaluated by dobutamine echocardiography. The median of follow-up after completion of therapy was 11,5 (range 4,7-15,2) years.Their ECGs were normal in basal conditions. They were 4 males and 5 females; 8 patients had localized tumor, 1 pt. multiple lung metastases; their localizations were extremities in 5, ribs in 3, vertebrae in 1; the median age at diagnosis was 8,2 (range 5,4-12,8) years; the median age at evaluation was 22,5 (range 12,8-25,6) years.
The **local treatment** was surgery in 4 pts., radiotherapy in 5 pts. (median 5170 cGy, range 4400-6000). No pt. had been irradiated on cardiac area. About **chemotherapy**, pts. had been treated with various protocols in different periods; they had received cycles of vincristine, cyclophosphamide, actynomycin-D and doxorubicin at standard doses. The doxorubicin doses were between 300 to 485 (median 428) mg/mq, administred bolus or in 30-60 min., generally using schedule of 3 consecutive days, intermittently, every 3 weeks in induction, every 9 weeks in maintenance. The median lenght of chemotherapy was 17 (range 8-21) months.
Methods- The Eco-doppler study was performed baseline and soon after two consecutive periods of dobutamine infusion (5 minutes at 5 gamma and 10 minutes at 10 gamma/kiloweight/minute). Besides heart rate (HR) and blood pressure (BP), some indexes of left ventricular function and end systolic wall stress (ESWS) were evaluated, according to adriamycin dose administred (< and $\geq$ 450 mg/mq), age at diagnosis (< and > 8 years) and lenght of follow-up after completion of therapy (< and $\geq$ 11 years).
Results- As expected, dobutamine increased systolic BP and decreased diastolic BP, with no significant changes in HR. Despite the overall increase in left ventricular contractility, as showed by progressive increase in left ventricular fractional shortening and ejection fraction values with dobutamine, a decreased septal systolic thickening was observed after inotropic stimulation(baseline 10±1 mm; 5 gamma: 10±2 mm; 10 gamma: 11±3 mm; p=n.s.) opposite to normal left ventricular posterior wall contractility (baseline:11±2 mm; 5 gamma:13±2 mm; 10 gamma: 15±3 mm; p=0.0001) and progressive reduction of left ventricular ESWS (baseline: 77±20 gr/cmq; 5 gamma: 67±16 gr/cmq; 10 gamma: 55±16 gr/cmq; p=0.0001).
Moreover, patients treated with adriamycin doses $\geq$ 450 mg/mq showed baseline left ventricular volumes (EDV baseline 126±13 mmc vs 100±12 mmc, p<0.05; ESV baseline: 50±3 mmc vs 35±7 mmc, p<0.01) and ESWS (baseline: 91±11 gr/cmq vs 66±17gr/cmq, p<0,05) greater than pts. treated with doses < 450 mg/mq and septal systolic thickening percentage significantly lesser with maximal (10 gamma /kg/min.) dose of dobutamine ($\geq$ 450 mg/mq: 45 ± 21%; < 450 mg/mq: 82 ± 24%, p<0.05).
Age at diagnosis and time elapsed from treatment withdrawal do not appear to significantly influence parameters considered.
Conclusions- In asymptomatic pts. with baseline normal left ventricular function, even many years after adriamycin therapy withdrawing, it is possible to show impaired left ventricular segmental contractility with pharmacologic stress tests. The data, if confirmed from larger studies, could represent a marker of long term cardiac effect of antracyclines.

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LATE FUNCTIONAL RESULTS IN CHILDREN AND YOUNG ADULTS WITH MULTIMODAL TREATMENT OF BONE TUMOURS

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With the increasing number of patients surviving disease-free following the diagnosis of malignant bone tumours and the availability of different modalities of local therapy and limb salvage procedures late functional results and quality of life measurements have become essential. The functional evaluation score of Enneking for Orthopedic sequelae, the Lent-Soma Scale for Radiotherapy sequelae, and quality of life measurements (EORTC QLQ -C 30) are being determinated in a cohort of patients with musculo-skeletal tumours surgically treated in a single institution (Münster) over the last ten year period and analysed according to data of first line therapy and complications. To date data of 80 patients from the total cohort of 350 patients are complete. The diagnose are: Osteosarcoma 35 pts., Ewing's-sarcoma 28 pts., Chondrosarcoma 9 pts and other musculoskeletal tumours 9 pts. The median age was 15 years (range 3-34). 47 pts were male. Median time since diagnosis was 40 months (range 14-24). The major primary sites were: femur (34%), pelvis (16%), tibia (15%) and humerus (11%). All patients had undergone surgery, 37/80 patients in combination with chemotherapy, 29/80 pts had also received locoregional radiation. The surgical procedures included amputation, rotation plasty, tumour resection and reconstruction with either endoprothesis, allograft or autograft.

Of 80 patients with complete data, the mean functional status scored 25 of a maximum of 30 (range 9-30). Only one patient required constant pain medication. 13 patients have been unable to return to their previous profession mainly due to long hours standing or a manual labour.

The given questonaire and examination profile allows differential functional assessment of different local therapy approaches and will helps to provide future guidelines.

P-253

LATE EFFECTS ON THYROID AND GONADAL FUNCTIONS AFTER THERAPY FOR CHILDHOOD MALIGNANCIES

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Parallelly with the improvement of the therapeutical results in treatment of childhood malignancies, a number of post therapy complications in patients' endocrine system are observed. In this study the function of the thyroid and gonadal glands in children having a longer than 3 years remission, following the anticancer treatment, is investigated. The thyroid function of 57 children was evaluated through T3, T4, TSH and TRH levels (ELISA) and through TgAb and Mab (haemaglutination test).
The gonadal function was verified by checking the levels of LH, FSH, Oestradiol and Testosterone pre- and post- Pregnyl administration (q 1000 IU for 3 days). Twenty three girls and 21 boys (age ranging between 8 and 19 years) were followed up.
Normal thyroid function was found in 51.1%, compensated hypothyroidism in 36.2%. Four patients had elevated levels of TgAb and Mab, 2 pts. had a weak response to THR stimulation.
A primary hypogonadism was found in 47.05% of boys and in only 23.52% in girls.
The compensated hypothyroidism was detected in children having radiotherapy in head, neck and chest regions. Those patients with chemotherapy alone, had no any evidence of thyroid damage. Again the radiotherapy in pelvic region in girls is associated with primary hypogonadism, while those treated with cytostatics alone, had no malfunction of the gonads. In boys 87.5% had primary hypogonadism after abdominal radiotherapy and 12.5% after treatment with alkylating agents.

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RENAL FUNCTION FOLLOWING IFOSFAMIDE WITH OR WITHOUT CISPLATINUM IN PATIENTS (PTS) WITH OSTEO-SARCOMA (OGS) (N=26) AND OTHER SARCOMAS (N=9)

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We analyzed the renal function in 35 pts, median age 16 yrs (range 7-20) who were treated with 72 gm/m^2 Ifosfamide (Ifos) without Cisplatinum (CDDP) (N=18) or 54 gm/m^2 Ifos with 360 mg/m^2 CDDP (N=17) at least 4 months following completion of chemotherapy. Group 1 had 9 pts with OGS who also received Adriamycin and high dose Methotrexate (HD MTX) and 9 pts with other sarcomas who also received Vincristine, Cyclophosphamide, Doxorubicin (Doxo), and Etoposide in addition to Ifos. Group 2 pts all had OGS and also received HD MTX and Doxo in addition to Ifos and CDDP. Median age and gender did not differ between groups. Results were as follows.

Parameter	Grp 1 (Ifos) Mean	Range	Grp 2 (Ifos/CDDP) Mean	Range	Nl. Range
GFR (inulin cl) (ml/min/SA)	100	73-150	91	71-125	90-130
RPF (renal plasma flow) (ml/min/SA)	470	371-762	450	319-579	300-700
FE glucose (%)	0.2		0.1	0-0.5	< 1%
FE phosphate (TMP/GFR)	3.3	2.7-4.0	3.4	2.1-4.8	2.5-6.0
24 hr amino acid excr.	2/18 pts slightly increased		2/17 pts slightly increased		
FE Mg (%)	4.8	1.67-10	5.2%	2.8-10.8	3-5
24 hr urine protein (mg)	102	22-473	118	16-319	<150
24 hr urine Ca (mg/kg)	3.9	2.7-6.8	3.3	1-8.1	<4
FE urate (%)	19	12-39	15	8-29	7-10
Serum HCO_3	Normal in all		Normal in all		--

In both groups, the somewhat low GFR's and elevated FE urate were the most consistent abnormalities found. However, the lowest GFR was not different in the two groups and none of the abnormalities observed would result in dose adjustment of any routinely used drugs. In conclusion, 72 gm/m^2 Ifos without CDDP or 54 gm/m^2 Ifos with 360 mg/m^2 CDDP do not result in clinically significant nephrotoxicity within several months of chemo completion.

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FINE MOTOR PROBLEMS OF CHILDREN TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Objectives: To describe in detail motor skills and handwriting performance of children after treatment for ALL.

Methods: 17 children were studied 2 years after cessation of treatment for ALL according to Dutch Childhood Leukemia Study Group protocols VI and VII. Treatment did not include cranial irradiation. At the time of the study children were 7 to 12 years old and the test results were compared with age- and sex-matched healthy controls (n=17). Gross and fine motor functioning was tested using the Movement ABC (Henderson, Sugden, 1992). Handwriting, a specific fine motor skill, was studied with the BHK, a 5-minutes during simple writing test, and a computerized writing task (XY digitizer; sampling rate: 142 Hz; RMS accuracy: 0.1 mm).

Results: Children treated for ALL performed worse on the Movement ABC as compared to their matched controls. This was most pronounced in fine motor skills. With regard to the quality of handwriting they also performed worse compared with the control group: specifically they showed more bad letter or word alignment, jerky handwriting, inconsistent letter size and ambiguous letter forms.
No significant group differences were found on the computerized writing task although individual children had problems performing this task.

Conclusions: Survivors of childhood ALL experience fine motor function loss and handwriting problems for at least 2 years after cessation of treatment.

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CISPLATIN DOSE INTENSITY AND NEPHROTOXICITY IN OSTEOSARCOMA

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Increasing the dose intensity (DI) of chemotherapy may improve the cure rate for osteosarcoma (OS), however this may also increase toxicity. The relationship between DI of cisplatin and nephrotoxicity has been investigated in patients with OS. Patients received 6 courses of cisplatin (100 mg/m^2/course) and doxorubicin (75 mg/m^2/course) either 3 weekly, standard dose intensity (SDI) -9 patients, or 2 weekly with GCSF support, high dose intensity (HDI) -8 patients. The serum magnesium (SMg) and the glomerular filtration rate (GFR), calculated by ^{51}Cr EDTA clearance, were measured 1 month, 6 months and then annually after the completion of treatment. Mean total dose of cisplatin was 583 mg/m^2 and 604 mg/m^2 for the SDI and HDI groups respectively. The mean DI was 38 mg/m^2/week in the HDI group and 27 mg/m^2/week in the SDI group (p= 0.004). One month after treatment mean SMg in the HDI group was 0.5 mmol/l (normal range 0.71-0.93 mmol/l), and in the SDI group was 0.61 mmol/l, p=0.056. There were no significant associations between DI of cisplatin and SMg or GFR at any other time point. These results confirm the clinical impression of increased renal tubular loss of magnesium and other electrolytes during treatment in the HDI group, but with no increase in acute glomerular toxicity.

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SIDE EFFECTS AFTER COMBINATION THERAPY FOR EWING'S SARCOMA: RESULTS FROM ONE INSTITUTION

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Purpose: With the improvement of treatment results in Ewing's sarcomas after combination chemoradiotherapy and surgery, the evaluation of (late) side effects (LE) becomes an issue of increasing importance.
In order to evaluate the scope of LE after "modern" combination treatment pts. having been treated in a single institution were over 15 years reloculated.
Patients and Methods: 22/47 pts. with Ewing's sarcoma (7-25, median 15.5 years) - treated between 1975 and 1990 - were alive 1/91 with a median follow-up of 46.5 mths. Specific follow-up examinations (median 29 mths. after end of treatment) for the detection of late effects (LE) could be carried out in 17 patients. Combination therapy included chemotherapy (VACA/VAIA), surgery and radiotherapy with doses ranging from 36-62.4 Gy. Functional and cosmetic results were investigated: skin, soft tissue, bones, joints; lungs, liver, kidneys, gonads. Extremity LE were categorized according to Jentzsch (1981) in "minimal", "significant", and "severe".
Results: Continous local control was archieved in 36/47 pts. (77 %). In extremitiy sarcomas 4/11 pts. presented with minimal LE (e.g. atrophy less than 1cm), 5/11 pts. with significant LE (e.g. limb shortening 2-3 cm), 2/11 with severe LE (pathological fractures, ostemyelitis, amputation/arthrodesis). 3/11 showed up with a valgus deformity of the ankle (<20°). In thoracic tumors 2/4 pts. revealed a marked scoliosis and a significant impairment of lung function. In pelvic tumors the LE were mainly dependent on primary site and surgical procedure. Impairment of kidney function was found in 3 pts. including 1 pt. with Fanconi syndrome (tibia sarcoma). From 11 pts. investigated (>12 years), elevated FSH levels were found in 4/7 males, amenorrhoe in 2/4 females. One patient died from ANLL 1 year after the end of combination treatment.
Conclusion: Compared with LE reported in literature, reflecting high dose radiotherapy as main local treatment, the pts. in this series generally presented with minor morbidity after combined local treatment. In future, further tailoring of the treatment volume of radiotherapy to the target at risk for local recurrence represents a major challenge to reduce LE without jeopardizing excellent local control.

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CARDIOTOXICITY AFTER ANTHRACYCLIN THERAPY AND PILOT STUDY FOR ITS PREVENTION WITH DEXRAZOXANE (CARDIOXANE)

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The late cardiotoxic effect of anthracycline therapy was studied by clinical and echocardiographic examination. 56 children were examined 0,5 - 8,0 (mean 2,5) years after the end of chemotherapy. 36 children had acute lymphatic leukemia (ALL) and 20 children osteosarcoma (OS). The range of the dose of anthracycline was 250 - 500 mg/m^2. 5 children with ALL and 5 children with OS had cardiac symptoms. Based on the clinical importance of antracycline related toxicity we performed a clinical investigation to study the efficacy and safety of the cardioprotective agent dexrazoxan. 13 patients with ALL received dexrazoxan (Cardioxan) and were compared with 13 children matched by age, prognostic group of leukemia and treatment modality. No adverse effects were observed after the infusion of Cardioxan except a slight, transitory local reaction in one patient. The remission rate, survival-, relapse rate and the early cardiotoxicity were not different in the two groups. We concluded that Cardioxan can be safely used in children, however, the observation period is yet short (9-18 month; mean 12.) to evaluate the late cardiac effects too.

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EWING'S SARCOMA OF LIMBS IN PEDIATRIC PATIENTS: LOCAL TREATMENT SEQUELAE.

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The **aim** of this study was to evaluate functional results and complications, in patients (pts) affected by localized Ewing's Sarcoma (ES) of the extremities, after different local treatment: surgery (S) vs surgery and radiotherapy (S+RT) vs radiotherapy alone (RT).
Patients: Since July 1980 to December 1993 among 56 pediatric pts with at least 24 months relapse free survival, 40 affected by ES localized to the limbs (lower limbs 34) were evaluated in this study. Sex: 25 males and 15 females; median age at diagnosis was 11,7 (range 0,9-13) yrs; median age at evaluation was 16,1 (range 5-27) yrs; median follow-up after completion therapy was 3,6 (range 1,2-15) yrs. One adjuvant combined protocol (protocol A) and two consecutive neo-adjuvant protocols (B and C) were used. The local treatment was: S in 27 pts (amputation in 4 and rotationplasty in 1), S+RT in 7 pts, RT in 6 pts. The median dose used for conventional RT (10 pts) in A and B protocols was 5200 cGy (range 4000-6000 cGy) and 6000 cGy (range 4800-6080 cGy) for accelerated and hyperfractionated RT in C protocol (3 pts).
Methods: Functional results were evaluated according to Enneking's criteria 2, taking into account seven primary factors (mobility, pain, stability, deformity, strength, emotional acceptance, functional activity). Each of these factors was defined as excellent, good, fair or poor. Because of the small number, pts were divided in two groups: pts with excellent or good functional results vs pts with fair or poor results, for each type of local treatment. Only major complications were considered: limb length discrepancy >5 cm, hypotrophy of irradiated tissue, joint subluxation, severe infection, breakage of instrumentation, pseudoarthrosis.
Results: Good-Excellent functional results reach the highest incidence in pts treated only with RT 83% (5/6 pts) vs S 59% (16/27) and S+RT 43% (3/7 pts). Major complications resulted more frequent (50%) in pts treated by RT only (limb length discrepancy > 5 cm 1 pt, hypotrophy of irradiated tissue 1 pt, joint subluxation 1 pt) while the remaining two groups had a comparable complication rate: 37% for S (limb length discrepancy > 5 cm 4 pts, severe infection 3 pts, breakage of instrumentation 1 pt, pseudoarthrosis 2 pts) and 43% for S+RT (joint subluxation 2 pts, pseudoarthrosis 1 pt). In most of the cases, complications in the surgical group were successfully managed or manageable (still on treatment) without worsening functional results.
In conclusion even if functional results are proportionally worse in S and S+RT group, S remains the main goal for treatment of these children, especially to avoid risks of secondary malignant neoplasm related to RT and to reduce the incidence of local recurrence.
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GROWTH AND ENDOCRINOLOGICAL STATUS UP TO 20 YEARS AFTER TREATMENT FOR ACUTE LYMPHOBLASTIC LEUKAEMIA IN CHILDHOOD.

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AIM. 1. To study variations in height standard deviation score (HSDS) and body mass index standard deviation score (BMISDS) from diagnosis up to 10-20 years (median 14.8 years) following the start of treatment for acute lymphoblastic leukaemia (ALL) in childhood. 2. To evaluate the endocrinological status at follow-up in these patients.
PATIENTS AND METHODS. Thirty patients (15 females and 15 males) treated for childhood ALL in the period 1973-1984 were investigated. Nineteen received chemotherapy and cranial irradiation with 24 Gy (C+I), while 11 received chemotherapy only (C). HSDS and BMISDS were calculated from the height and weight data at diagnosis, after end of treatment and at follow up 10-20 years (median 14.8 years) after diagnosis, using age and sex matched normal material.Provocative growth hormone (GH) tests (clonidine- and insulin tolerance test) and ACTH test were performed. Blood samples were drawn for analysis of T-4, T-3,TSH, prolactin, FSH, LH, estradiol (girls), and testosteron (boys).

RESULTS. In the C only group mean HSDS was at the same level at follow-up and at diagnosis, while mean BMISDS was steadily increasing from diagnosis until follow-up ($P<0.002$). In the C+I group mean HSDS was steadily decreasing from start of treatment until follow-up ($P<0.001$), while mean BMISDS was continuously increasing ($P<0.001$) Nine patients in the C+I group were GH deficient, while 2 patients in the C only group were GH deficient. Two patients had hypogonadism and one patient had hypothyroidism.
CONCLUSION. Growht suppression and obesity were observed at long-term follow-up in patients treated with C+I for ALL in childhood, while only obesity was observed in those treated without cranial irradiation. Except for GH deficiency endocrinological deficits were rare.

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GONADAL DAMAGE FOLLOWING TREATMENT FOR MALIGNANT LYMPHOMAS IN CHILDHOOD

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To evaluate long-term gonadal toxicity of therapy for malignant lymphomas in childhood, we studied 99 pts treated for Hodgkin's Disease (HD) and Non-Hodgkin's Lymphoma (NHL). Limited data is currently available on gonadal function of NHL pts.
PATIENTS: -HD: 46 subjects (34M, 12F), age at diagnosis 8.6±3.3 yrs, 26 treated with MOPP±ABVD or COPP±OPPA and 20 with ABVD. All pts had radiotherapy (RT) (12 below the diaphragm). **-NHL:** 53 subjects (44M, 9F), age at diagnosis 7.6±3.4 yrs, treated with different protocols (LSA2-L2, COMP, TotalB2) including cyclophosphamide (CTX) (from 1.2 to 19.8 g/m^2). Basal and peak LH and FSH were measured (LHRH test, 50μg iv) in all pts, 5.0±2.6 yrs after therapy. The results were compared to those of 296 control subjects with the same Tanner pubertal stage; values above 97th centile were considered abnormal. At diagnosis 81 pts were prepubertal and 18 in puberty; when examined 25 were prepubertal, 18 in pubertal stage II-III, 56 in stage IV-V.
RESULTS: Females: only 1/21 females (a NHL patient in pubertal stage II-III, the only female submitted to abdominal RT) showed high gonadotropin values.
Males: prepubertal (6 HD, 14 NHL) and **pubertal stage II-III** (9 HD, 8 NHL) males showed normal mean gonadotropin values. **Pubertal stage IV-V** males (19 HD, 22 NHL) showed mean basal and peak FSH values significantly higher than controls ($p<0.05$ and $p<0.01$ respectively for HD and NHL), NHL showed also a significantly higher mean LH peak ($p<0.01$). High FSH values were seen in 7/19 HD pts (36.8%): i.e. in 7 of the 12 pts treated with alkylating agents and 0 of the 7 treated with ABVD (58.3% vs 0%, $p<0.02$). Only 2 of these 7 pts had also abdominal RT.
Among the 22 NHL pts 11 (50%) had high FSH values: 1 pt had testicular RT, 3 had CTX at a dosage <10 g/m^2 and 7 >10 g/m^2 (respectively 30% and 63% of the pts treated with analogous doses).
CONCLUSIONS: 1) females are more resistant to gonadal damage induced by chemotherapy for lymphoma; 2) the prepubertal testis is already sensitive to chemotherapy-induced damage which will be apparent only with the progression of puberty; 3) ABVD regimen seems to be less lesive for the testis; 4) the risk of testicular damage is similarly high in boys treated for NHL or HD (with alkylating agents).

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HEARING LOSS (HL) IN CHILDREN AND YOUNG ADULTS RECEIVING HIGH-DOSE (HD) CISPLATIN (DDP) FOR THE TREATMENT OF OSTEOSARCOMA (OS)

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Background: DDP is effective in the treatment of several pediatric solid tumors, including OS. Nephrotoxicity and HL limit its clinical use, however. We undertook a prospective study to determine extent of HL in pts with OS receiving HD DDP.
Method: Between 5/88 and 5/94 22 children and young adults 8 to 19 yrs (median 13.5) with OS received 3 to 8 courses of DDP (median 6) from 90 to 160 mg/m2/course (median 120). Cumulative doses (Σ) ranged from 400 to 1040 mg/m2 (median 720). Each pt underwent pure tone audiometry (PTA) at diagnosis, prior to each course, and ≥ one month after the last course of DDP.
Results: All baseline PTAs were normal. The number of pts abnormal (≥ 15 dB loss from baseline at 4000-8000 Hz or ≥10 dB at 2000 Hz) versus number of patients tested at ΣDDP is presented in Table 1.

TABLE 1. **# OF PATIENTS ABNORMAL/# OF PATIENTS TESTED**

ΣDDP DOSE (mg/m^2)	2000 H_3	4000 H_3	6000 H_3	8000 H_3
400-480	0/22	1/22	2/22	2/22
520-560	0/20	0/20	0/20	1/20
600-640	2/18	3/18	3/18	3/18
720-760	1/14	4/14	4/14	4/14
800-840	0/8	3/8	4/8	4/8
920-960	1/4	2/4	2/4	2/4
1040	0/1	1/1	1/1	1/1

Grade (gr) 0 HL (<40 dB loss at all frequencies) was seen in 5 pts at Σ560-960 mg/m2, gr 1 (≥40 dB at 8000 Hz) in 7 pts at Σ480-840 mg/m2, gr 2 ((≥40 dB at 4000 Hz) in 9 pts at Σ600-1040 mg/m2, and gr 3 (≥40 dB at 2000 Hz) in 1 pt at Σ 750 mg/m2. No gr 4 (≥40 dB at 1000 Hz) was seen.
Conclusions: HD DDP was commonly associated with mild (gr 1) and moderate (gr 2) HL. Severe HL (gr 3-4) was infrequent. Overall 77% of pts had HL at 8000 Hz, 72% at 6000 Hz, 63% at 4000 Hz and 18% at 2000 Hz. HL was not seen with <600 mg/m2, but at 4000-8000 Hz HL was noted at 400 mg/m2. This study suggests that HD DDP is associated with considerable HL of a mild to moderate degree but that severe HL is uncommon.

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ECHOCARDIOGRAPHIC ASSESSMENT OF THE LEFT VENTRICULAR ACTIVITY IN CHILDREN TREATED WITH ANTHRACYCLINES FOR LEUKAEMIAS

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Anthracyclines belong to a group of drugs which may cause abnormalities of the left ventricular (LV) function and lead to an irreversible dialated cardionyopathy. Despite such in an undesirable side effect anthracyclines are still applied due to their effectivness in the treatment of cancers. That is why a periodical an systematic assessment of the LV with the use of an non-invasive method is required.

The assessment of the cardiac activity of 41 children with leukemia was made. The children had been treated with anthracyclines and had their treatment discontinued in the course of a long-term remission (group I). Observation time since the therapy cassation varied from 3 to 12 years. The average dose of anthracyclines was 350 mg/m2. No significant changes have been found in the physical examination.

Control group included 30 children whose age, gender, body weight and cardiac activity were similar to these from the study group (group II).

The average value of the LV ejection fraction (%EF) was similar to the results obtained in healthy children and was 62% and 66%. The mean value of the circular fibre shortening fraction (%SF) in children treated with anthracyclines was lower than that (38%) observed in healthy children. When analyzing particular cases of children treated with anthracyclines in 22 % of children the lowering of the %EF was observed and the %SF index was determined in 24% of children.

Children treated will anthracyclines were found to have the diastolic function impaired, what was manifested with a significant decrease in the velocity and deceleration of the LV diastolic inflow as well as a decrease in the mean value of the inflow acceleration and delay.

The most sensitive parameter of the LV function impairment in children treated with anthracyclines turned out to be deceleration of early LV inflow through the mitral valve.

Children treated for leukemia who had been treated with anthracyclines should have their cardiac function systematically controlled. Doplers echocardiograpy provides a non-invasive assessment of the LV systolic and diastolic function.

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RADIONUCLIDE VENTRICULOGRAPHY (RV) IN MONITORING ANTHRACYCLINE CARDIOTOXICITY IN PATIENTS WITH OSTEOSARCOMA (OS)

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Doxorubicin (DOX) is an effective drug in the treatment of OS, but its use has been limited due to cumulative dose-dependant cardiac toxicity. To determine the cardiac changes produced with a DOX chemotherapy based regimen, the left ventricular ejection fraction (LVEF) was measured by (RV), according to the guidelines of Children Cancer Group (CCG). Thirty six patient (pts) with OS were treated since October 1991 with chemotherapy: 3 cycles of DOX 75mg/m^2 by 24 hours IV infusion and Ifosfamide (IFO) 2gr/m^2/days 2-6 every 3-4 weeks followed by Cisplatin (CDP) 4 cycles preoperatively, and 2 to 4 after surgery. The sex ratio was 1:1, with median age of 14y (range 6-19). The total cumulative DOX dose administrated was 300mg/m^2 to 525mg/m^2 (mean 418,7mg/m^2). RV were obtained at baseline, at 225mg/m^2, at the end of therapy and at yearly interval. The RV falled significatively in 16 of 36 pts (44.4%): in 13 pts during treatment and in 3 post therapy. Only 3 pts recovered. One girl (2.8%) developed a congestive cardiac failure after a total dose of 450mg/m^2 with a fall in the RV to 25% and finally dead. Twelve pts (33.3%) are currently in follow up (6-51 months) with decrease in RV but asymptomatic. The RV is a sensitive noninvasive method, to detect the presence of preclinical myocardial deterioration and could be used for monitoring the systolic cardiac function in long-term survivors, mainly in those who had persistent low values of RV post therapy and to be on the alert of chronic myocardiopathy development. The actual usefulness of cardioprotective agents still need more support.

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GROUP MEETINGS OF YOUNG ADULT SURVIVORS OF CHILDHOOD CANCER

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One of the observed late effects of treatment in children for cancer are emotional disorders. These are probably the main causes of their unsatisfactory professional and social achievements. In order to help these young adults we arranged group meetings. The aim of these meetings was to give them the opportunity to share their common experiences about the cancer and its treatment, to talk about painful feelings, fears, coping styles, interpersonal relationships, education and professional future. In our first group participated 10 young adults and professionals (two doctors, the clinical psychologist and the nurse). We have had monthly meetings in a period of one year. After ten meetings the whole group spent one week in a spa for physical and psychological rehabilitation. After the completion of their program, we have evaluated the effects of rehabilitation regarding to their behavior and the emotional status. We have noticed among the members of the group much more spontaneous communications, more mature emotional reactions and more self-initiatives. The fear of the medical authority staff and disease itself were less present. They found themselves ways to help each other and some of them made a durable friendship. Because of the very good experience of the first and the second group, we have decided to proceed with the same program, and have now a third group. To collect more resources, we have established a foundation to support and help young survivors at their physical and psychosocial rehabilitation and better quality of life.

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THE ROLE OF THE PSYCHOLOGIST IN A PEDIATRIC ONCOLOGY UNIT - SUPPORTIVE GROUP / EXPRESSIVE GROUP PSYCHOTHERAPY AS AN EXAMPLE

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This study brings into discussion the integrated role of the psychologist in a Pediatric Oncology Unit. The activities developed by the psychologist at our unit consist of: (a) psychological support to all patients and family at diagnosis and at moments of crisis; (b) accompanying patients during invasive medical procedures and outpatient chemotherapy sessions; (c) individual psychotherapy (focal, brief or supportive); (d) coordinating supportive/expressive group psychotherapy; (f) participating in multidisciplinary meetings; (g) participating in weekly meetings with medical and nursing staff providing guidance in management of relationships among health personnel. The aim of this study is to discuss the supportive / expressive group psychotherapy. This intervention may help parents of children with cancer better manage relationships with family members, friends and health care personnel. At our Unit we run a self help group encouraging expression of illness related-emotions through the exchange of their experiences. This group is coordinated by a psychologist and includes members of the multidisciplinary team, parents and relatives of the patients. Group members meet weekly during one hour. Up to now we have recorded 50 group-meetings. The most frequent issues raised by the parents are: (*) disease-related feelings such as fear of not been able to cope with the illness; (*) treatment-related feelings such as expectancy and the importance of adopting a "fighting spirit"; (*) team-related feelings such as idealization of the supportive attitude of the health care team. Depression and self help were the most frequent functioning issues observed. The most frequently used defense mechanisms were denial and projection. We believe that this activities seem to help parents and relatives to achieve better compreension of topics involving the disease as well as to relieve their anxiety. In addition to its assistential activities the psychologist should play and active role as a facilitatur within the multidisciplinary team. However, the influences and benefits of the psychologist activities for the child and family care as well as for the health care team could be better evaluated on a controlled study.

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CANCER DEATH IN OFFSPRING

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Cancer clinically manifests itself suddenly in apparently healthy children. Initial symptoms are usually aspecific, and the diagnosis is reached while parents are still unprepared to face a life-threatening illness. Many parents report a sense of losing unlimited potential. Prolonged treatment, long-term recovery, pain, follow-up protocols—all these may be disruptive for the family. The most difficult issue that families must face is the loss of control over their own lives (daily organization, perception of family unity); whatever the outcome, families will always experience a great loss. Nevertheless, the death of a child shakes the foundation of a family. Many studies have been carried out on the quality of life in cancer patients during or after treatment, but, what do we really know about about the actual outcome of the family when a child dies? In the Oncology Service of the Pediatric Clinic of the University "La Sapienza" of Rome, we evaluated parental occupation and health, offspring, divorce, pregnancy, volunteer work, and types of charity in two different family groups having children affected with cancer. The first group comprised families with children who died of disease (F1: 48 families); the second group instead, comprised families with children who survived (F2: 34 families). Both were homogeneous for all the variables tested except for only one significant difference—the number of new pregnancies after the death/diagnosis of the child ($p<0.05$, OR = 3.71). In twenty-one of 38 evaluable families with childhood cancer deaths (55.3%) in which the mother's age was less than 45 yrs, the mothers became pregnant—15 of these (71.4%) less than one year after the death. In F2 instead, new pregnancies were only 8 of 33 cases (24.2%). The replacement child syndrome, which refers to a child who is used as a substitute for a sibling who has died, could explain this difference. The replacement child is often expected to emulate the idealized image of the dead child, and is therefore not allowed to develop his or her own identity. Replacing a child with another allows the parents to partially deny the first child's death; however, this can act as a barrier to the parental acknowledgment of death. Thus, parents should be counselled by the oncologist and/or pediatrician about the dangers of having another child before resolving and overcoming their grief.

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PSYCHOLOGICAL ASPECTS IN PAEDIATRIC OSTEOSARCOMA.

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Osteosarcoma is the most common bone malignant tumor in paediatric oncology (5% of total paediatric tumors). It appears in adolescence and requires chemotherapeutic and surgical treatment.

In our Centre we follow all types of malignancies and we have a routine psychological support based on individual non structured interviews; so we have been able to observe the psychological aspects and needs in osteasarcoma compared with leukemia, that is the most frequent paediatric tumor.

Since 1984, when neoadjuvant treatment started, we observed 78 osteosarcoma patients aged from 1 to 19 years, and 225 acute leukemia patients aged from 1 to 18 years.

In osteosarcoma, the physical changes (tumor mass, loss of limbs through amputation, scars, ...) can affect the adolescent body image, and often are felt as a deterioration of self both at physical and mental level.

Adolescence is a special period, a time of transition from infancy to adult that involves physical, emotional and social growth and may be more difficult and painfull than it is, due to bone tumor onset. It seems that, together with the physical inability and the fear of "not standing on your own two feet", a deep anxiety of mental dependence can develop. Furthermore the consequences of surgery cannot be hidden or forgotten, thus hindering the use of denial, which, in adolescents with leukemia, is the most frequent psychological defence mechanism employed. The request of individual psychological support has been greater in the group affected by bone tumor than in the other at the disaease onset, and less frequent after the end of the therapy.

The early psychological intervention facilitates the working through and the acheiving of an adequate coping strategy.

The group of leukemia patients, instead, is not so available to think of disease axperience, and, in a certain sense, is at risk of developing later anxieties.

Due to our knowledge, we stress that the osteosarcoma patients should have psychological support at diagnosis. Finally, we should take into account that different specialists (oncologist, radiologist, orthopedist, vascular surgeon, ...) are involved in osteosarcoma treatment, which can cause the risk of the splitting of the patients.

Eventually, a good communication should be manteined to make the patient feel receiving a global care.

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PROMOTING RESILIENCE IN CHILDREN WITH CANCER

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Pediatric psycho-oncology, recently included in the Psychology Health Care program, implies specific intervention strategies to avoid dramatization of the word Cancer and associated myths related to treatment and prognosis, and improve the quality of life of our patients.

Being aware of the diversity and complexity of a child's feelings in relation to health professionals, or family and friends who surround them, and taking into consideration that each child reacts differently when faced with adversities, the authors did a study of Resilience ("Human capacity to overcome adversities" -Edith Grotberq) -in children with cancer, aiming to find strengths that exist within the individual child and his/her environment in order to build upon them and also to identify the child at risk. Method: - 36 children between the ages of 8 to 12 years were interviewed and distributed equally as to their socio-cultural level (high, medium, low). The interview was based on the 3 essencial sources of Resilience: I am, I have and I can. Results: The study demonstrated that most children have the capacity to overcome adversity related to severe illness and hospitalization. All children presented features related to all 3 sources of resilience but in this age group the parameter I have was predominant. Children from medium/low socio-cultural levels achieved resilience quicker than those from a higher level. Professionals as well as family members have an important role to play in promoting resilience. Conclusion: Most children do achieve resilience but others are at risk, professionals have an important role to play in identifying "at risk" situations, especially family disfunction. Help cannot be sectorial, it must be personal. Professionals must help child and family develop their own resilience features.

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LANGERHANS' CELL HISTIOCYTOSIS (LCH) IN CHILDREN: THE ISTITUTO NAZIONALE TUMORI OF MILANO EXPERIENCE

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Sixty-five consecutive patients (M/F:43/22) with LCH were diagnosed between 1975 and 1994. Their median age was 5 yrs (range 0.6-17) and 26 were aged < 3 yrs. Thirty-five pts had a single localized lesion (stage IA according to Raney and D'Angio classification) affecting bones (33) or soft-tissues (2). Twenty-four had multifocal disease (stage IB) affecting only bones (13), or bones ± skin ± ear canal ± liver enlargement (11). The remaining 6 pts had multifocal LCH plus organ dysfunction (stage II) involving lungs (2), liver (3), liver and bone marrow (1). In pts with localized disease treatment consisted of watchful waiting (4), surgical excision ± radiotherapy (24), chemotherapy alone (7). Management of patients with multifocal disease consisted of radiotherapy (1), chemotherapy (16), or both (7). Drug therapy was used for all pts with organ dysfunction, in combination with RT in 3. The drug regimen included Vincristine, Ciclophosphamide, Prednisone for 6-12 mos; doses of radiotherapy ranged from 6 to 15 Gy. Recurrence was observed in 21/65 children: 8 (22%) of stage IA, 8 (33%) of stage IB, and 5 (83%) of stage II, after a median time of 4 mos (range 2-25). Among pts with only bone involvement, the relapse rate was 14%. Of the children < 3 yrs, 50% failed. Two pts (Stage IB and II, respectively) died of progressive disease, whilst all the other children of this series remained alive in CR. The 5-yr survival rate was 97% (median follow-up 110 mos, range 17-249). Late sequelae related to disease or treatment consisted of permanent diabetes insipidus in 5, and second tumors in 2 (thyroid adenoma and intra-abdominal desmoid tumor). In conclusion, young age (< 3 yrs) at diagnosis, site of involvement other than skeleton alone and organ dysfunction correlate with a higher recurrence rate. However, the prognosis of LCH remains excellent.

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ALPHA INTERFERON AND RETINOIC ACID FOR THE TREATMENT OF PEDIATRIC NEUROENDOCRINE TUMORS: MOLECULAR, PRECLINICAL AND CLINICAL STUDIES.

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Alpha interferon (IFNα) is used in a variety of tumors of adulthood, but scarce data is available on the use of IFNα in the treatment of pediatric malignancies. It exerts its effects through specific cell surface receptors.
We studied the expression of IFN type I receptor (IFNr) in 20 solid tumors of childhood, including neuroblastoma, PNET, Ewing's sarcoma and RMS, using the IFNα receptor-specific antibody IFNaR3 and the APAAP technique.
All tumors expressed IFNr, although at different levels. No relationship between expression and histology was observed. From three of the tumors, a neuroblastoma, a PNET and a Ewing's sarcoma, we obtained continuous cell lines. Cell lines were used to study the structure of the receptor by affinity cross-linking and immunoprecipitation of ^{125}I-IFNα-IFNr complexes with IFNaR3 and an anti-IFNα MoAb.
Receptors showed a multichain structure composed of a 110 and a 90 kDa IFNα binding proteins, the latter of which was specifically recognized by IFNaR3. IFNα receptors were capable of mediating an antiproliferative effect in the 3 tumor cell lines studied and their expression was up-regulated following treatment with trans-retinoic acid (ATRA). The association of IFNα and ATRA showed a more potent inhibition of tumor cell growth than each single agent, in vitro. We also studied the effect of IFNα alone or in combination with ATRA in a xenograft model of human PNET in athymic mouse. Statistical analysis showed that IFNα/ATRA combination treatment was more effective than each single agent in inhibiting tumor growth in vivo, although both substances demonstrated some antitumor activity. Electron microscopy of treated tumor xenografts showed neuronal cell differentiation when treatment included ATRA.
Based on these results and on the pharmacokinetics of ATRA, we are conducting a phase II clinical trial in order to evaluate the efficacy of IFNα and trans-retinoic acid for the treatment of neuroendocrine tumors of childhood.
This project was supported by a grant from AIRC.

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MALIGNANT ENDOCRINE TUMORS IN CHILDHOOD AND ADOLESCENCE - RESULTS OF A RETROSPECTIVE ANALYSIS

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Background: Malignant endocrine tumours (MET) are rare neoplasms in childhood and data on such tumours are scarcely available. The aim of this retrospective study was to collect data over a period of 11 years (1984-1995), giving the basis for a prospective study in Germany.
Patientes and methods: 180 departments of paediatrics, nuclear medicine and children's surgery were asked for reporting of patients with MET by questionnaire.
Results: 35 % of the departments answered. 112 children with MET were reported: 81 with thyroid cancer (52 papillary, 17 follicular, 1 medullar, 1 anaplastic; 1 : 1,9 boys to girls, both mean and median age 11 ¾ years, 14 with adrenocortical cancer and 17 with other malignant endocrine tumours.
Metastases were found at diagnosis in 42 of 69 children with papillary/follicular carcinoma. 42 patients (pts.) are in first continuous complete remission (CCR), 4 in partial remission (PR) and in 23 (!) children the remission state or the outcome is not known. 10 of 11 children with medullary cancer have had metastases at diagnosis. 2 pts. are in CCR, 4 pts. are living in PR, 1 in relapse and 1 patient is alive with unknown state of remission. 3 children with metastases at disease diagnosis, died.
In 13 pts. with adrenocortical cancer, the sex-ratio was 1 : 1,3 (boys to girls; mean age 5 ½ years, median age 2 ¾ years). All tumours showed hormonal activity, 6 children disclosed metastases at diagnosis. All patients were treated by surgery, 8 received chemotherapy additionally. 6 children are living in CCR, 2 pts. in remission of unknown state, 4 died (all of them with metastases at diagnosis), 2 pts. are lfu.
The other MET reported: 14 carcinoids (13x appendix, 1x lung), 2 phaeochromocytomas, 1 islet cell cancer. 11 pts. are in CCR, 1 is alive in unknown state of remission and 4 are lfu. The child with the islet cell carcinoma died.
Conclusions: Since only 35 % of the clinics answered, this retrospective analysis cannot give any statement about the incidence of MET in children. As to the outcome, thyroid cancer seems to have a favourable prognosis in childhood and adolescence. In contrast, metastatic adrenocortical cancer is incurable in this age group. Carcinoids of the appendix can be treated curatively by appendectomy. To better understand the biology of MET in children and adolescence and to achieve a better prognosis for some types of these tumours, much more data are needed. For this reason a multicenter prospective therapy study for childhood MET seems to be necessary.
Key-words: thyroid carcinoma, adrenocortical carcinoma, endocrine tumours, childhood.

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CHILDHOOD HEMANGIOPERICYTOMA

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Purpose: Hemangiopericytoma is a very unusual tumour in children with no significant, large experience available. The purpose of this study was to assess the optimal treatment modality in management of these tumours.
Methods: Between 1969-1994, 5 children aged under 10 years were treated in a single institution. All medical records, details of site of primary tumour, surgical, other modalities of treatment and microscopic sections, follow-up data were reviewed.
Results:

Patient No.	Age	Sex	Site of Primary	Mode of Presentation	Therapy	Recurrence /Metastasis	Follow up (mo)
1	1d	M	Rt. forearm	Mass	OP	-/-	A&W (36)
2	28d	F	Rt. orbit, maxillary middle fossa	Mass + bleeding	OP+CTX	-/-	AWD (12)
3	4yr	M	Abdominal mass	Acute abdomen + bleeding	OP+CTX +XRT	+/+	DOD (18)
4	7yr	F	Lt. axilla	Mass	OP	-/-	A&W (84)
5	10yr	F	Rt. scapular area	Mass	OP	+/-	A&W (60)

OP - Operation; CTX - Chemotherapy; XRT - Radiation Therapy, A&W - Alive & well; AWD - Alive with disease; DOD - Dead of disease; RT - Right; LT - Left; D - Day; Mo - Month, Yr - Year

Three out of the five tumours had features of malignant hemangiopericytoma. Surgical resection was incomplete in these three patients. Two of the three patients survived.
Conclusions Biological behaviour of the tumour is difficult to predict. Complete wide local excision should be the treatment of choice, although incomplete excision is still compatible with a good prognosis. The role of radiotherapy and chemotherapy is not well defined. Congenital tumours seem to respond better than other tumours.

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CURE OF INFANTILE MYOFIBROMATOSIS WITH SERIOUS RESPIRATORY COMPLICATIONS WITHOUT ANTITUMOR THERAPY

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Infantile myofibromatosis (IM) has two clinical courses - death, if vital viscera are involved, or spontaneous regression of the lesions and survival without sequelae.
Case history: A six weeks old boy presented with a pathological femoral fracture. One month later he developed multiple tumors of the skull, malaise and weight loss. Radiology at the age of 3 1/2 months revealed multiple osteolytic lesions in the skull, scapula, ribs, pelvis and long bones. Biopsy showed the typical picture of myofibromatosis. No visceral involvement was found.
Complications: Hypercalcemia was treated with calcitonine. Respiratory failure due to osteolytic ribs (analogous to a "flail chest") demanded mechanical ventilation for eight months. In the same period the osteolytic defects progressed and did not improve until 1 1/2 year after diagnosis. Chronic malnutrition required enteral feeding by gastrostomy (PEG).
Therapy: In spite of the rapidly progressing disease and serious complications only supportive therapy was given, relying on the good prognosis reported in the literature in cases of IM without visceral involvement.
Follow-up: The lesions subsided gradually leaving slight deformities but normal function. At the age of 3 1/2 years the boy has an excellent quality of life.
Conclusion: Our case illustrates how extensively the lesions can progress and still resolve with time.It stresses the importance of high quality intensive and supportive care.

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CLINICAL CHARACTERISTICS OF SMALL FUNCTIONING ADRENOCORTICAL TUMORS In CHILDREN

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To characterize the clinical and pathologic findings and outcomes of children with small functioning adrenocortical tumors, twenty of sixty-seven children, registered on the International Registry of Childhood Adrenocortical Tumors between May 1988 and December 1994, were studied. All had small adrenocortical tumors (defined for this study as measuring ≤ 200 cm^3 and/or weighing ≤ 100 grams. The median age was 2 years (range, 4 months-5 years). There was only one boy.All had clinical signs of virilization, and seven had signs or symptoms of Cushing syndrome. A median 5.5 months (range, 1-40 months) had elapsed between the first signs of endocrine dysfunction and diagnosis. All tumors were surgically resected. Tumor volume was 3.3-195 cm^3 (median, 38.7 cm^3) and weight was 3.7-100 g (median,36 g). Tumorsamples were histologically reviewed In 18 cases. Eight were adenomas and ten were carcinomas (6 low and 4 high-grade). Pathology records diagnostic of adrenocortical carcinoma were available for the remaining two patients. One patient received mitotane for 8 months after surgery. Only one patient had recurrent disease, wich was detected 6 months post-diagnosis and proved rapidly fatal. Another has been lost to follow-up. The remaining 18 patients are alive with no evidence of disease at a median 2.3 years (range, 6months-6.1 years) after diagnosis. Our data suggest that children with small functioning adrenocortical tumors have an excellent prognosis with surgery as the sole therapy, regardless of tumor histiotype.

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LANGERHANS CELL HISTIOCYTOSIS (LCH) IN CHILDREN. A RETROSPECTIVE EVALUATION OF 30 CASES.

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Langerhans Cell Histiocytosis (LCH) is a proliferative disorder of unknown etiology characterized by the accumulation of abnormal histiocytes. Even if LCH is a malignant disease, the clinical course is unpredictable, varying from benign cases with spontaneous regression to very aggressive ones.
We describe here the experience of Hemat. Div. of "Bambino Gesù" Hospital, which includes 30 cases (median age 40 months, range 2-131)evaluated between January 1980 and October 1995.
In 21 pts (among 30) the pathologic diagnosis was based on the presence of characteristic Langerhans cells and their S-100 positivity. Other 9 pts was diagnosed by the basis of morphologic evidences on bioptic studies coming from other Institutions, for diagnostic confirmation. In 10 pts (since 1986) the original diagnosis was confirmed by Electron Microscopic studies.
According Histiocyte Soc. Classification, the patients are classified: 12 pts group I, 6 pts group II, 4 pts group III, 8 pts group IV.
Nine pts (group I) received, as treatment, only surgical "curettage", 2 (group II) curettage plus immunotherapy (thymic extract), 1 (group II) curettage plus Vinblastine, 5 (group I, II, III) Vinblastine, 6 (group II, III, IV) VP-16, 6 (group III, IV) polichemotherapy (ADR, VCR, CY, PDN or VBL, CY, MTX, PDN), and one only thymic extract. After first line treatment, 20 pts (66.6%) reached I CR, 1 is on treatment, 5 were Non Responders (NR), 4 died for disease progression.
Three pts relapsed 5,5,39 months after I CR and has been successfully retreated with Vinblastine, being actually in II CR.
Among the 5 NR pts, 2 are on treatment, and 3 obtained a I CR after a long period of a second line polichemotherapy. Two of these relapsed after 2m and 6m, but reached a II CR (one) and a III CR (other) with monochemotherapy (VP-16).
The overall survival was 85% at 187 months and the Disease Free Survival is 72% at 170 months.
The prognostic factors are discussed. The monochemotherapy even if it is certainly adequate at onset, however has a role also in the subsequent relapses; therefore is discussed the usefulness of polichemotherapy, still in consideration of new therapeutical approaches.

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PROSPECTS OF PEDIATRIC ONCOLOGY DEVELOPMENT IN ARMENIA

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Two years ago at SIOP meeting in Paris I reported on state of pediatric oncology service in Armenia. Hard economic condition of our Republic didn't allow to hope for any improvement of treatment quality of children's cancer. But great desire to help every sick child and everyday labor let us overcome the crisis. For today the Pediatric Oncology Unit for 20 beds functions in a normal way. The contingent of patients has increased , including by hospitalization of the children with lymphomas. The Hodgkin desease and nonhodgkin lymphomas of abdominal localization are the most frequents. There are no diagnostic errors. The greatest difficulties are those with antitumor drugs and antiemetics. New hope appeared as a result of collaboration with Saint-Louis hospital in Paris, the specialist s of which render assistance in organization of treatment of Hodgkin desease according the protocol MDH-90. Practical study of our specialists has already began, the supply of medicines is in the offing. I'm sure that optimism and a friendly support is a powerful strength against the children's cancer.

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MALIGNANT NEOPLASTIC MORBIDITY IN PEDIATRIC POPULATION IN OREL REGION

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The paper analyses malignant neoplastic morbidity, nosologic structure and mortality in pediatric population of the city of Orel and its region over 10 years after the opening of the pediatric consulting room at the Cancer Hospital. The data indicate the appearance and increase of Thyroid cancer morbidity among pediatric population after 1986, what is connected with radionuclide contamination, as a result of the Chernobyl accident. Now the rate of growth of the Thyroid cancer morbidity in Orel region is 13 times more than all over Russia in average.

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THE EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF CHILDHOOD MALIGNANCIES IN THE PAEDIATRIC CLINIC OF RIJEKA, CROATIA

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The aim of the study was to analyse malignant disease's incidence in our region; western and littoral parts of Croatia with 598691 inhabitants of which 111016 are children under the age of 16. For time being, there is no population based registry of childhood malignancies in Croatia. We made a retrospective-prospective analysis of hospital cases of childhood malignancies in the period from 1971-1995, and compared it with German Registry of Childhood Malignancies(*). Paediatric Clinic of Rijeka had diagnosed and treated 360 cases of childhood malignancies in last 25 years. Diagnoses were certified by pathological and cytological laboratories of Medical faculties of Rijeka, Zagreb and Ljubljana. All cases were classified and re-examined according to Birch & Marsden Classification of Malignant Diseases (1987). Patients were followed up from max. 25 years to min. 6 months. The results of our study showed almost identical distribution of disease spectrum, incidence of single diseases and disease groups, age and sex distribution as those presented in German Registry. Hospital based analysis has shown to provide a reliable information about relative incidence of malignancies in children, with awareness of its limitations. It also gives a basic ground to start a regional registry of childhood malignancies to improve consistency of registration, patients follow up, treatment quality and results, side effects, secondary malignancies and social adaptation of treated patients.

(*) The epidemiology of childhood malignancies
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RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA: STUDY OF FREQUENCY, PRIMARY SITE AND SURVIVAL IN A PERIOD OF 15 YEARS

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In Jan 96 we studied 325 children treated during the period Jan 80 to Dec 92. 218 children were treated from Jan 80 to Dec 92 with one Brazilian Cooperative Protocol (80, 82 or 85 protocol) and 107 patients received the BFM protocol (Jan 89 to Dec 92). In Jan 96 all patients were 5 years or more off therapy with the Brazilian and 1 year or more with the BFM protocol.
115 patients had one relapse, 56 had two and 11 had three:

Sities of relapsed

One relapse (115)		Two relapses (56)	Three relapses (11)
BM	63	39 (22 after BM, CNS 6, Test 3, Iris 3,Other 5)	6 (1 after BM+CNS-CNS, 1 BM-BM, 3 BM-BM+CNS, 1 Test BM)
CNS	22	09 (2 after BM, CNS 2, Test 3, BM+CNS 2)	3 (2 after BM-CNS, 1 CNS-BM+CNS)
Testi	12	01 (After BM)	
Iris	05		1 (After Iris-BM)
Mediast	01		
BM+CNS	09	07 (3 after BM-CNS, 1 Iris)	1 (After BM+CNS-CNS)
BM+CNS+Test	01		
Iris+CNS	02		

21 patients are alived (18% survival): 14 with one relapse (6 after Testicular relapse, 5 after CNS, 1 after Iris, 2 after Bone Marrow); 3 of them are during treatment and the other 11 are off therapy 7 years or more. From the 21 alived patients 7 had 2 relapses (1 BM/BM, 1 BM/Testis, 1 BM/CNS, 1 BM/Iris, 2 Testis/CNS, 1 Iris/BM); 4 are 6 years off therapy or more and 3 are receiving treatment.
In this study we analised the relapses with: frequency, period of treatment, laboratorial data and clinical envolvment at diagnosis.

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THE RESULTS OF ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT IN ACCORDANCE WITH BFM-90 PROTOCOL (1991-1995)

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25 patients (pts) aged 2-13y (14 m and 11 f) with acute lymphoblastic leukemia underwent BFM-90 protocol treatment in 1991-1995. The variant c-ALL was determined in 68% of cases (17 pts),Ia-like - in 12% and T-cell - in 16%. Biphenotypic variant was in 1pt. According to risk groups as defined by the protocol criteria there were SRG - 13 pts(52%), MRG - 5 (20%), HRG - 7(28%). There were no changes for risk groups during treatment. Every pt had positive response to prednisolone treatment by the 8th day of therapy. The remission was achieved in all pts after the protocol first phase (33-d day). The peculiarity of protocol conduction was the use of methotrexate in the dosage of 1g per square meter in protocol "M". The 5-years survival was 80% .20% of pts died: 2 pts in SRG during prorotocol "M",protocol 2. One pt in HRG had primary resistant course of the disease. 2 pts (1 from HRG, 1 from SRG) died due to early development of relapse 1-5 months after the completion of active therapy. All these 5 pts died during the first year of treatment. There are 20 pts in the state of complete remission now. 4 pts undergo various stages of active therapy, 5pts receive supportive therapy while the treatment was abolished in 11 children.

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IMMUNOPHENOTYPE OF BONE MARROW LYMPHOCYTES: pre-B-CELLS OF PATIENTS WITH AND WITHOUT LEUKEMIA.

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We have investigated bone marrow (BM) lymphocytes(lph) of 23 patients(pts) with c-ALL: 5-before treatment and 18 in the remission (3 - 5 yr) after the protocol BFM - 90. We defined the percent of cells with "leukemic" immunophenotype CD10+ CD19+ HLA-DR+ and the intensity of expression of these markers, CD20 and CD22 molecules. For this purpose we used Leukemia Research and IMK Plus Kits of MCA, FACStrak, Simulset and PS-LYSYS programmes (Becton Dickinson). The results have been compared with the same data of 8 pts without leukemia. In this pts it was 12-25% of lph, expression CD10+ CD19+ HLA-DR+ was 5-15%. In pts with c-ALL in the remission it was 9-27% of lph; among them the number of cells with "leukemic markers" was 18-67%. Analysis of the intensity of expression showed the high level of CD 19 in comparison with CD 10 in all cases of leukemic and nonleukemic pts. It was revealed the difference of expression CD20 and CD22 membrane markers of these cells. We try to use this difference to evaluate "real" leukemic cells. No relapses were diagnosed in pts in remission. There was no correlation between the number of cells with CD10+CD19+ phenotype and remission duration.

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Treatment of pediatric B-NHL with different modes of therapy.

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30 children (47 % males) aged from 2 to 14 years with B-NHL have been treated from November 1980 to July 1995. Before 1988 15/30 pts. were treated with surgical resection and cyclophosphamide, vincristine and low dose methotrexate (Group I) and after 1988 15/30 with chemotherapy alone with B- NHL BFM protocols 86-90 (Group II). According to Murphy's classification : 4 pts were Stage II (13%), 22 pts. Stage III (74%), 4 pts, Stage IV (13%).

	Estadio I	Estadio II	Estadio III	Estadio IV
Group II	0	4	10	1
Group II	0	0	12	3

In Group I all pts. had abdominal disease, 1 also had bone marrow involvement. In group II the most common primary site was abdominal involvement 12 pts. (80%) , 1 with SNC disease; 3 pts. (20%) had lymph nodes on head and neck, 2 also had bone marrow involvement . Ten pts. in Group I and all pts. in group II went into complete remission (CR). Failures were all in Group I : 3 surgery related sepsis, 2 refractory diseases and 4 relapses , one of them is alive en 2 nd CR. All the relapses were before 8 months of diagnosis.
The 5 year event free survival was 57 % for group I vs. 100 % for Group II (p = 0.0016).
Conclusion : A significant difference in outcome was found between pts. diagnosed prior and later 1988. B-NHL can be cured with intensive hemotherapy protocols. It is unnecessary to perform ablative surgery.

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OSTEOGENIC SARCOMA (OS) IN CHILE. REAL RESULTS INCLUDING ALL PATIENTS AT RISK

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200 OS patients has been treated from 1969 to 1995. Since 1987 HLCM is the national center for the OS treatment. ALL THE PATIENTS ARE INCLUDES INDEPENDENT IF THEY ABANDON THE TREATMENT, REFUSE THE SURGERY OR THE CHEMOTHERAPY, ARE SECOND MALIGNANCY, INITIAL METASTATIC OR MULTICENTRIC .
4.5% are 3-5 years old, 22.6 % 6-10 y, 60.4% 11-15 y and 13.5 % over 16 . Male are 53%.The distribution is : femur 59.4%, tibia 23.6%, humero 9.4%, sacro/pelvis 3.1%, fibula 1%, maxila 1 %, clavicule 0.5 %, costal 0.5% , metacarpe 0.5%, multicentric 1%
The initial lung metastases has increased from 9 to 20%.
Chemotherapy has been: 1) VCR/ACTD/CTX, 2) MTX/ ACTD/CTX, 3)DOX combinations, 4) and 5) CDDP/DOX, 6) CDDP/DOX/IFO and 7) CDDP/DOX/IFO/HDMTX.

	1	2	3	4	5	6	7
Years	**69/74**	**75/76**	**77/79**	**70/83**	**83/87**	**87/92**	**92/95**
total	**9**	**9**	**13**	**7**	**46**	**64**	**48**
% alives	**0**	**33**	**31**	**71**	**50**	**47**	**76**
%limb sparing	**0**	**0**	**0.7**	**0**	**13**	**20**	**42**
%amputation	**11**	**44**	**8**	**43**	**37**	**48**	**19**
%desarticulation	**56**	**44**	**77**	**57**	**33**	**17**	**19**
%no surgery	**33**	**11**	**8**	**0**	**13**	**6**	**20**
%metastasectomy	**0**	**11**	**23**	**14**	**4**	**22**	**17**
alive after metast.	**0**	**1/1**	**2/3**	**1/1**	**1/2**	**7/14**	**4/8**

Conclusion: The great majority of OS in the last years in Chile are included. Without any exclusion we observe a real improve in survival and quality of life.
12/21 patiens are long survivors after metastasectomy (1992).

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IFOSFAMIDE - DOXORRUBICIN / H.D. METHOTREXATE AND LIMB SALVAGE SURGERY A ALTERNATIVE IN OSTEOSARCOMA TREATMENT

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Between July 1.991 to September 1.995, 27 patients under 18 years with Osteosacoma were admitted in SA Hospital of Children J. M. de Los Ríos and in Hospital Oncológico Padre Machado in Caracas, Venezuela. All them were selected for a study with Ifosfamide- Doxorrubicin and HD Methotrexate neoadjuvant chemotherapy and Limb Salvage Surgery protocol. M : F sex ratio was 0.92 :1.70. 39 % had age between 6 and 11 years , the median age was 10.74 years (min. 4y, max 18 y.) The most common primary sites were : Femur 62.97% Tibia 22.23 %. 2 pts. had metastatic disease at diagnosis. In all patiennts , the diagnosis was established by Pathology : Conventional Osteosarcoma and histologic varietis 92.59%(25 pts) , Telangiectasic Osteosarcoma 3.70 % (1 pt) and Small Cell Osteosarcoma 3.70% (1 pt). 22 pts were evaluable for therapy. 91 cycles of chemotherapy wereadministered. Complete Response (CR) was obtained in 77.28 % (17 pts) and Partial Response (PR) in 22.72 % (5 pts.) In 7 patients limb salvage surgical was performed , and radical surgical in 10 patients .11 pts. (50 %) are alive , of them 9 (81.81 %) are free of disease. 8 patients relapse : 5 Pulmon, 2 Bone, 1 Pulmon and Bone, of them 3 had limb salvage surgery , only one had local skin relapse . 11 patients died : 8 in relapse and 3 for HDMTX toxicity. Free Disease Survival was 51 % at 36 months, and the Overall Survival was 52 % at the same time. **Conclusion** : Ifosfamide / Doxorrubicine + HD MTX combination associated to limb salvage surgery constituted *a alternating* schedule for Osteosarcoma treatment, but , because the great volume tumoral that present our patients at diagnosis , the poor nutricional conndditions of the same , the low social-economic conditions and the development of metastasic disease early , kept digits of survival lowered. Our results are similar to reported in other countries in development.

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USE OF COMPRESSION-DISTRACTION METHOD IN COMPLEX OF TREATMENT OF MALIGNANT LOCALISED BONE TUMORS.

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Since 1991 eight children with malignant bone tumours of different localisation have been treated in the Children's Hospital of the Archangelsk Medical Academy. Two of them, a girl of 13 and a boy of 13, had tumours located in the knee joint area. At the primary biopsy osteosarcoma of the femur was diagnosed in the first case, and malignant fibrotic histiocitoma of the tibia in the second one. In the preoperative period 3 cycles of chemotherapy (CT) (in case of osteosarcoma: Adriamycin 30 mg/m^2/day x 3, Cisplatin 150 mg/m^2/day x 1; in case of histiocitoma: Vincristin 1.5 mg/m^2/day x 2, Cyclophosphamide 300 mg/m^2/day x2, Doxorubicin 30 mg/m^2/day x2) and radiation therapy of the tumours were administered to each of the children. The operation consisted of segmental resection of the bone involved, with the articular cartilage being left intact, of osteotomy and osteosynthesis using the Ilizarov device. Subsequently, both patients received 7 cycles of CT; at the same time repair of the segment defect was performed by bone regenerate distraction and formation, as well as by achievement of consolidation. It was achieved 10.5 and 12 months after the operation, respectively. The Ilisarov devices were removed 11 and 12.5 months after the operations, respectively. By now the postoperative follow up periods make 48 and 54 months; both children can put their weight on the affected limb, but the shortening by 2.5 and 3 cm, respectively, is observed.

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PEDIATRIC RHABDOMYOSARCOMA IN A DEVELOPING COUNTRY : İSTANBUL EXPERIENCE

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Between 1980-1995 48 children (30 males,18 females) with rhabdomyosarcoma (RMS) were treated in our center. Their initial evaluation were made according to TNM and IRS groups. The median age was 5 years (4-16 years). 45.8% of the patients were <5 years of age while 41.8% were between 5-10 years and rest (12.4) >10 years. Primarily tumor localization were head and neck (31.25%), genito-trinary (22.9%), extremities (10.4%) trunk (8.33%) and the others (27,12). The histopathologic distributions was mainly embrional (81.25%); only 8.33% was alveolar and 8.33 undifferentiated and 2% other. Our patients were mainly in stage III (47,9 %) and IV (25 %) which is very characteristic for all solid tumors in developing countries due to late admission. The patients were treated according to IRS I,II,III protocols changing in years. Primary total tumour resection could only be possible in 35.4% of the patients. Unfortunately 70.8% of the patients were lost to follow up, 85,3% during chemotherapy and 14,7 %in later stages. Six patients died and 17 (35.4%) relapsed (median 4 months). Only two of the relapsed patients still lives under treatment, the rest either expired or lost of follow up, (median follow up 8.5 months). The 5 year overall survival was found to be 74.4% and EFS 43.83%. Only sex and age was found to be effective for survival.
As a result,although our overall outcome was surprisingly high.the high numbers of the patients living therapy or lost to follow up is still a problem to be solved to make a more realistic evaluation.

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A POSITIVE TREATMENT STRATEGY IN RHABDOMYOSARCOMA

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From May 1993,to November 1995, our medical team lived a big and successfull challenge. A young girl 14th years old, was diagnosed to have, more than 20 cm , embrional rhabdomyosarcoma, in the retroperitoneum.In collaborative group studies , for regional disease where complete remission is achieved with chemotherapy alone or combined with surgery or radiotherapy for minimal residual disease,five years survival should approach 70%. We decided to begin the following chemotherapy combination: Vincristine,Adryamycin,Cisplatin,Ciclophosphamide,Dactinomycin(North American IRS Committee,Regimen 37A). Definitive local therapy according to the protocol was approached at 20th week of treatment when surgery was done.The patient was operated with an internal hemipelvectomy avoiding the use of screws and plates, so what no artifacts would later misdiagnosed in MRIs. So we decided to use Fibula autograft,thus facilitating a limb preserving procedure.The pathologic final result demonstrated: Iliac bone: 16X12x6cm,medullary fibrosis and chronic infection,without neoplasia. Soft tissue: conjuntive tissue and muscle with calcification foci, without neoplasia.
The end of the treatment was on Nov. 1995, with alternated pulses VAdrc with VAC.Until now the patient is felling well,without any sings of active disease and with good psychological and emotional conditions.

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IMMUNOPHENOTYPE ANALYSIS RHABDOMYOSARCOMA IN CHILDREN AS RELATED TO THEIR SURVIVAL IN CONTINUOUS COMPLETE REMISSION

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Abstract:

24 cases of rhabdomyosarcoma (16 rhabdomyosarcoma embryonale, 2 sarcoma botryoides, 2 rhbadomyosarcoma alveolare, 2 rhabdomyosarcoma pleomorphicum and 2 mesenchymoma malignum) were studied to evaluate phenotype of proliferating cells. Paraffin - embedded tissue sections were stained by the immunoperoxidase method to demonstrate vimentin (Vi), desmin(Des), myoglobin (Myo) and actin (Act) using DACO antibodies.

Four groups of phenotype were selected:group I (Vi+ , Des-, Myo-, Act-) in 8 cases (6embryonal ,2 mesenchymoma malignum), group II (Vi+, Des+, Myo-, Act-) in 7 cases (6 embryonal ,1 sarcoma botryoides), group III (Vi+, Des+, Myo+ , Act+) in 5 cases (2 embryonal, 2 alveolar , 1 sarcoma botryoides) and group IV (Vi-, Des+, Myo+, Act+) in 4 cases (2 embryonal , 2 pleomorphicum).

Desmin and vimentin staining was widely distributed.Myoglobin and actin staining was in the larger differentiating tumour cells only.

Survival in continuous complete remission children were studied as related to phenotype of RMS cells.These studies showed that rhabdomyosarcoma in children express immunohistochemical markers for intermediate filaments and muscle proteins in a cumulative sequence with morphological differentiation and resemble normal myogenesis. Some primitive tomours of rhabdomyoblastic nature expressed vimentin alone and may be the couse of diagnostic mistake.

Patients who belonged to group I, II and IV have showed good response to therapy. Survival in continuous complete remission was : 62,5%, 71,4% and 100% respectively. Patients in group III, represent the most abnormal phenotype all had bad response to therapy and survival in continuous complete remission in that group was only 40%.

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The Radiation Accident at Kiisa

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On Nov.17, 1995, came 13-year-old boy to Tallinn Children's Hospital with blisters and necrotic lesions on his hands, subfebrile body t^{o} and epistaxis. Because of his complaints he was suspected of radiation injuries. There was found a radioactive source (Caesium-137) in his home kitchen, the dose rate at the surface of the source was $2\text{-}3x10^{5}$ R/h. Several members of the family suffered from radiation sickness, one died. The retrospective estimate showed that the boy had an intermittent exposure between Oct.21 up to Nov.17, a daily exposure about 9-10cGy. After the admission to the hospital there occured a severe decrease of all blood cells, DNA damage, chromosomal aberrations, changes in immunological status. Histologically the bone marrow showed a transient aplasia. Retrospectively the bone marrow's recovery showed a typical case of a chronic radiation exposure. Because the experience and signs and symptomatology were compatible with a spontaneous recovery of hemopoiesis cytokines were not used.

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B HEPATITIS, EPSTEIN-BARR VIRUS AND HIV INFECTION ACCOMPANYING HEMATOLOGIC MALIGNANCIES IN CHILDREN

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We report some relations between hematologic malignancies and chronic virus infections in children treated in a single unit.

The patients were 26 children aged 1-15 years (median 6 years 8 months) treated between 1991-1995 for: ALL-14, AML-4, Hodgkin's disease 4 and NHL-4 patients. Serological markers for HBV (HBsAg Monolisa), EBV (Paul-Bunnell reaction) and HIV (ELISA +/- Western Blot) were studied at the time of diagnosis and every 6 months until the last follow-up in January, 1996.

Eleven children were HBsAg positive (42%) at the time of diagnosis: 8/14 ALL, 1/4 HD and 2/4 NHL patients. One male, five years old (3,8 %) had evidences of both AIDS and immunoblastic NHL st IV. Heterophil antibodies suggesting recent infection with EBV were positive in 3 newly diagnosed patients (11,5%): one AML-M2 13 year-old male, one lymphocyte predominant HD st IIIB 5 year-old female and one lymphoblastic NHL st IV 3 year-old female.

Early death was recorded in 8 patients (30%), closely related to the type of malignancy (4/4 AML, 3/4 NHL, 1/14 ALL, none HD) and 3 of them were infected: NHL+AIDS, AML+EBV and NHL+EBV+HBV.

In January, 1996, 14 patients (53,8%) were alive in complete remission (median survival 25 months). Only two were HBsAg positive, meaning a surprisingly high rate of conversion. None had evidence of EBV or HIV.

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LOW DOSE ANTHRACYCLINE THERAPY CAUSING CARDIOMYOPATHY IN CHILDREN

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Anthracycline administration is associated with dose related cardiomyopathy. We have been arbitrarily limiting the dose of anthracycline from 450-500 mg/m^2. No studies are available from India so as to ascertain whether western data regarding this is applicable to our population. We have undertaken a prospective study to monitor the cardiac function of patients receiving anthracyclines. Sixteen children (14 boys and 2 girls) with malignancies (9 ALL, 5 lymphomas and 2 retinoblastoma) aged 6 months to 12 years receiving anthracycline therapy were evaluated by baseline chest x-ray, electrocardiogram and 2 D echocardiography. The tests were repeated after 180-200 mg/m^2 of anthracycline dose. The left ventricular ejection fraction fell from a mean of 66% (range 57-77%) to 58% (range 38-75%) after 180-200 mg/m^2 anthracycline (P<0.01) Fractional shortening also fell significantly from 0.40 to 0.34 (P=0.0174). There was no significant difference in systolic time interval, cardiothoracic index or ECG findings. Out of 16 patients 4 (25%) developed significant deterioration of cardiac function according to the recommendations of cardiology committee of children cancer study group (>10% fall in ejection fraction) and required discontinuation of anthracycline therapy. Two of these 4 patients were symptomatic and developed frank congestive heart failure requiring digoxin therapy. The ejection fraction of these four patients improved after discontinuation of anthracycline but deteriorated again on restarting anthracycline in two of those in whom it was restarted. This small study indicates that incidence of cardiomyopathy due to anthracycline is significant despite using low doses of these drugs.

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REVERSE ISOLATION IN PEDIATRIC ONCOLOGY: SENSE OR NONSENSE?.

Name(s) of author(s), institution, city, country:
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Abstract:
The policy regarding reverse isolation in pediatric oncology, in particular with respect to patients with long periods of neutropenia, e.g. following AML induction therapy, differs widely between pediatric oncology centres and between the different countries. Research directed towards establishing the significance or futility of the different reverse isolation procedures seems to be lacking. From the nursing point of view, we are anxious about the negative effects of strict reverse isolation measures for the affected child regarding psychosocial isolation from family members other than parents, siblings, and friends. In addition, we are worried that the isolation barrier may prevent the isolated child from appropriate nursing attention. It was therefore decided that we, as a group, would want to stimulate thinking and research in order to be able to provide rational and sound guidelines towards appropriate nursing care for patients in nadir periods, respecting the need for protection as well as the child's and family's need for psychosocial support and communication. Different models as in current use in centres we are representing will be presented.

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ALLOGENIC BONE MARROW TRANSPLANTATION AT THE WILHELMINA CHILDREN'S HOSPITAL

C.G.M. Brokke, University Hospital for Children and Youth, Het Wilhelmina Kinderziekenhuis, Department of Immunology and Hematology, Utrecht, The Netherlands

At the department of Immunology and Hematology allogeneic bone marrow transplantations are performed in children with certain immunological and hematological diseases. In our hospital these children are undergoing a selective decontamination instead of a total decontamination. Those children undergoing a selective decontamination dont't have to be nursed aseptically. At our department such children are being nursed in a room with laminar down flow. Only if there is nobody else around in the room, the patient is allowed to walk freely by itself in the room. If not the patient has to stay in bed underneath the planem. This way of selective decontamination has been chosen by the staff of our department because we believe it to be less damaging psychologically for child and parent. For more information see photographs on poster.

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION.

A.Westerdijk and J. Theunissen
University Hospital Nijmegen, The Netherlands.

Autologous Peripheral Blood Stem Cell Transplantation (APSCT) is a new development in the pediatric oncology. Untill recently children with metastatic tumors or children with tumors orleucemia were treated with Autologous Bone Marrow Transplantation (ABMT) in the University Nijmegen.
We tried to reach a survival with only a small change of relaps or complcations. ABMT was a long treatment that resulted in a long isolation of the child. APSCT prommise to be less interfering. Faster recovery makes shorter days of hospitalisation, because of the platelet recovery.
This does not mean that the treatment is less intensive for the child.
Intravenous administration of high dosis of chemotherapy, before restitution of blood stem cells, *makes* the therapy intensive.
The poster presentation will explain you the nursing-care during Autologous Peripheral Blood Stem Cell Transplantation.

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HEMATOPOIETIC STEM CELL UNIT IN A PEDIATRIC BMT DEPARTMENT.

B Verny, R Eglizot, M N Mixy.
BMT dept, Hotel-Dieu Clermont-Ferrand, France.

Our pediatric BMT- unit was started in 1983 for children from Auvergne area. We have two laminar air flow rooms and we transplant patients with most frequently solid tumors and leukemias.

In 1991 a hematopoietic stem cell unit (HSC) was created within our pediatric BMT-unit. the objectives of this HSC unit are the following :
1) HSC collection from bone marrow, peripheral blood and from cord blood.
2) HSC concentration and cryopreservation.
3) Immunoselection using the cell Pro stem cell concentration system on peripheral blood cells in neuroblastoma in remission.
4) HSC infusion

Activities during 5 years from March 1991 to March 1996 are :

collection			Cryoconservation		selection	graft	
marrow	blood	cord	-80°c	- 196°c	CD34	auto	allo
43	360	18	110	320	32	68	30

The unit consists of : bone marrow harvest room; aphereses room; cryoconservation room and laboratory room. The experience base has been acquired through general oncology nursing supplement by special training.
The integration of the unit within the BMT deparment improves relation in general with important benefits for the children.

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ACUTE TOXIC LARYNGITIS (ATL) IN CHILDREN TREATED FOR BRAIN TUMORS WITH HIGH-DOSE CHEMOTHERAPY (HDC) AND AUTOLOGOUS BONE MARROW (ABMT) SUPPORT

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The aims of this study were to evaluate the incidence, the complications rate, the outcome and the risk factors of ATL observed in children treated for brain tumors with HDC and ABMT. Since 1988, 61 children were treated for brain tumors by combined high-dose Busulfan Thiotepa and ABMT. During their hospitalization in the BMT unit, 11 of them experienced ATL. Its median time of onset was day 10 post BMT (R5-23) and its median duration was 15 days (R4-24). In 9/11 children, ATL occured during the post BMT aplastic phase. In 8/11 children (73 %) ATL was followed by pulmonary complications, however in no instance mechanical respiratory assistance was necessary and no death related to ATL was observed. Symptomatic treatment consisted of combination of antibiotic, antifungal, corticosteroid and aerosol therapy.
In our experience, out of more than 600 ABMT performed in children, ATL was exclusively observed in children treated for brain tumors with Busulfan-Thiotepa combination. Wether this complication is related to the brain tumor and its neurologic signs, to HDC, or both, remains a major question and analysis of risk factors will be presented.
In conclusion, ATL is a rare and severe complication of HDC, exclusively observed in children treated for brain tumor. Pulmonary complication rate is particularly high (73 %) in this setting as compared to other patients (20 %). Due to acute respiratory disturbances, this complication is life threatening and needs intensive supportive care. After this study we tried different ways to administer aerosol therapy and finally we found more convenience and efficiency by using masks.

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INTRAVENOUS (IV) RECOMBINANT GRANULOCYTE COLONY-STIMULATING FACTOR (rhG-CSF) FOLLOWING MYELOSUPPRESSIVE CANCER CHEMOTHERAPY

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The approved routes of administration for rhG-CSF (Neupogen, Filgrastim) are IV and SQ, but limited evidence suggests that efficacy is greater with SQ administration due to prolonged serum rhG-CSF concentrations. This study was designed to determine if the pharmacokinetic parameters of IV rhG-CSF in children are comparable to results from previous studies of SQ rhG-CSF administration. 12 children were randomly assigned to receive 10 days of IV rhG-CSF at 5 or 10 μg/kg/day, beginning 24 hrs after completion of a course of myelosuppressive chemotherapy. The pharmacokinetic results demonstrated that IV administration compares favorably with that previously reported in children with SQ administration. The duration of therapeutic serum concentrations of rhG-CSF are sufficient to allow an adequate hematopoietic response. Therefore, the IV route appears a suitable method of rhG-CSF administration in children treated with myelosuppressive cancer chemotherapy. Since IV administration is more costly than SQ, and SQ administration is associated with a daily painful injection, the parents and child should be involved in the choice of administration method.

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INTRAVENTRICULAR CHEMOTHERAPY IN PEDIATRIC BRAIN TUMOR PATIENTS WITH MENINGEAL DISEASE

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Because of the limited penetration of systemically administered cytotoxic drugs across the blood-brain barrier treatment for patients with brain tumor dissemination to the leptomeninges is generally unsatisfactory. Recognition of this problem led to the development of new therapeutic strategies including direct intraventricular therapy via an indwelling subcutaneous reservoir. Over a 2-year-period 13 brain tumor patients (one to 19 years old) with cerebrospinal fluid (CSF) tumor dissemination received regional therapy over a Rickham or Ommaya reservoir. The intraventricular chemotherapy consisted of methotrexat 2 mg daily over 4 days or mafosfamide, a preactivated derivative of cyclophophamide, 20 mg weekly or biweekly. Because the Rickham or Ommaya reservoir is connected to an intraventricular catheter - and in shunt-dependent children also to a shunt - rigorous adherence to aseptic methods is necessary to prevent meningitis in these immunosuppressed children. The intraventricular therapy is administered over 5 to 20 minutes by the pediatricians with assistance of the oncologic nurses in the procedure room on our ward. We describe the role of the oncologic nurses in preparing the surgical tray, assisting the physician and monitoring the patient for adverse effects during and after therapy. Except for one minor Rickham infection which could be treated successfully by local vancomycin instillation no infectious complications occurred in 266 intraventricular drug administrations. Except for a headache and minor nausea in some patients the intraventricular chemotherapy was tolerated well. Since craniospinal irradiation has been associated with significant sequelae in the young child, adjuvant intraventricular therapy might be a promising alternative and eventually help to obviate the need for neuroaxis irradiation.

P-300

STANDARD NURSING PROJECT "ELEVATED INTRACRANIAL PRESSURE" IN CHILDREN WITH A BRAIN TUMOUR.

Mulder J, Weitenberg T, Sulkers E. Division of Pediatric Oncology, Beatrix Children's Hospital, University Hospital, Groningen, The Netherlands.

Introduction. In the Beatrix Children's Hospital of the University Hospital in Groningen, The Netherlands, a newly developed patient file is used in which the "nursing process" is included. In 1992 a study group was installed to investigate nursing problems and to develop matching nursing interventions. In the Department, which is also accomodating Pediatric Neurology and Pediatric Neurosurgery, the nursing standard "elevated intracranial pressure" is used in children with a brain tumour. By using a uniform language we hope to improve the communication in the group and towards other disciplines. As the complexity of care for children with a brain tumour is high and medical assistance is given multidisciplinary, standardizing is a practical expedient to obtain plans for individual nursing. Moreover the nursing standard "elevated intracranial pressure" means to achieve better quality of nursing care for children with a brain tumour.
Method. In the Beatrix Children's Hospital the model "primary nursing" is used. The nurse who takes care of the hospitalized child is also responsible for the nursing process. Thus the continuity and coordination of the total care is guaranteed. From the assessment the nursing problems will be listed, formulated and elaborated. The nursing standard "elevated intracranial pressure" is used to acquire an individual plan for a child with a diagnosed brain tumour. Using the evaluation criteria from this individual plan, reports will be made. If necessary the plan will be adjusted.
Conclusion. The nursing standard will stimulate the nursing process. By using univocal formulation the communication in the group and towards other disciplines will be improved. Finally the quality of care will be advanced by using the nursing standard "elevated intracranial pressure" in children with a brain tumour.

P-301

Our experience in work with central venous catheter in children oncologic patients

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In the period from 1990-1995 there were 126 permanent central venous catheter (51 Broviac, 40 Port a cath, 35 Groshong) implanted at the Oncology ward of the Children's Hospital Zagreb
In the study we present:
- advantages of central venous catheter in long, exhaustive chemotherapy in children- oncologic patients
- way of correct working with catheter in accordance with the rules of asepsis
- indications for particular sort of central venous catheter in relation to living condition (war circumstances, impossibility of heparinisation, obeying rules of asepsis in distant parts of country)
- necessity of correct determine of sort of applied catheter concerning patient ages, sort of applied chemotherapy, distance of place of living and equipment of local health institution
- our way of education of parents and staff which handles central venous catheter

The complications of central venous catheter in our patients (11 injuries, 9 septicaemia, 1 clotting) were also shown).
Concerning our experience in the our patients Groshong catheter (which need no heparinisation) are not convenient for implantation in smaller children due to their tendency for injuries in extra corporal parts. Due to war circumstances in which we are living- bad traffic connection with some parts of country are forced to apply more expensive Port a cath catheter because it demands less frequent heparinisation and can't be injured what enables children to stay longer at home.

P-302

COLLABORATIVE MODEL - COOPERATING WITH INSTITUTIONAL AND PRIMARY HEALTH SERVICES FOR CHILDREN WITH CANCER IN TRONDHEIM, NORWAY

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The Norwegian Cancer Society and the Pediatric Clinic in Trondheim have developed a model which promotes professional cooperation between the institutional and the primary health services in the districts of Mid-Norway (pop. 620.000). The main objective is to improve the quality of care for children with cancer and their families. It involves all health care professionals in institutional and primary health services, including schools, daycare centers, public health nurses and doctors in the patient's home communities. We have experienced that structured collaboration contributes to bridge the gap between these services in addition to promoting an effective working method for all health professionals.
The method used secures an overview of what each family with a child with cancer prossesses in the way of personal resources, as well as social networks and other forms of support. This knowledge is then applied to help in the care of each individual child with cancer and their families.
This service has been provided to children with cancer and their families since 1988. It has been shown that it can be usefully applied to other groups of patients as well.
The results of this collaborative model are being evaluated to further improve professional cooperation between the institutional and the primary health services, which again will improve the quality of care for children with cancer and their families.

Our poster will describe the methods we use in this cooperative model. We will also have an informative brochure explaining this model.

P-303

HELPING SIBLINGS COPE

Name(s) of author(s), institution, city, country:
Ramholt, Bente, clinical specialist nurse
The Norwegian Cancer Society
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Abstract:
When cancer strikes a child, the whole family is affected. Parents are mainly preoccupied with the sick child, a fact that usually leads to considerable change in the family's social structure and daily routine.
Siblings are at risk separated as they are from their parents, both psychologically and physically. The siblings often feel very lonely, and they may consider themselves very different from their peers, since none of them can quite understand their problems.
In order to alleviate the siblings feeling of being "the only one", of being put aside, of feeling guilt, worry, jealousy,anger etc. we have organized sibling-groups . These groups meet regularly to discuss their particular situation. The purpose is to increase their ability to cope through recognizing their own feelings in others, learning to express them and how to deal with them.
The group meetings are very popular with the sibling group (ages 7-12 years) and seem to be a useful tool in helping to normalize their lives.

P-304

A PRESENT WITH FUTURE "VAIXELL 92'"

Cristina Caixal, Alba Lloret, Carmen Pérez, Isabel Sánchez.
Pediatric Oncology Unit. HUMI VALL D'HEBRON.
Barcelona. SPAIN.

The treatment of children affected with cancer supposses a psychological and emotional impact to be taken into consideration by the nursing staff.
For this reason, our work brings the assistance service together with the psychological support.
The aim of our study is directed towards the achievement of a good adaptation of the oncologic child to the hospital enviroment and to favour a better tolerance to the treatment during the admission.
The oncology unit of our hospital provides a playing room and a library (Vaixell 92') which were opened in 1992. This allows the children to take part in different entertainment activities according to their ages and they can also enjoy it during admissions and outpatient treatments.
Through our experience in the service and the results obtained in the survey of a group of children, we can say that by means of the playing room a decrease in individualism and a chanelling of the agressivity through the play is achieved. It also favours the relationships between them and a better cooperation with nursing staff.
In conclusion, we see this experience as totally positive and believe that the creation of Vaixell 92' improves the adaptation of the oncologic children to the hospital environment and make a better tolerance to the treatment possible.

P-305

THE MODEL OF PSYCHOSOCIAL CARE-CONCEPT IN THE ONCOLOGIC DEPARTMENT OF THE ST. ANNA CHILDREN'S HOSPITAL IN VIENNA

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ST. ANNA CHILDREN'S HOSPITAL, VIENNA, AUSTRIA

ABSTRACT
An ultimately comprehensive treatment of children who suffer from cancer includes and maintains the integration of a psychosocial care-concept. The basic services of the system provide both the child and the concerned family with counselling. the key issues of the psychosocial care-concept employed in the St. Anna children's hospital are based on the principles of psychotherapeutic communes. Apart from the individual psychological counselling of the child and the family, an attempt is made to enhance the interpersonal competence of all persons involved in the medical treatment. Therefore we are coordinating the different activities of all staff members through regularly held group meetings. In addition, there are regular interdisciplinary group meetings with the medical staff to ensure that the coordination is appropriate. These meetings focus on the patient and his/her family as well as the interaction and the interpersonal relations between the patient, the family and the staff.
The foremost objective is to provide an optimal bio-psychosocial support.

P-306

A BUTTERFLY FLIES AWAY

Conny Molenkamp, pediatric oncology nurse,
Amsterdam, The Netherlands.

A butterfly
so beautiful, so full of life
that flutters around you
and shows you
the paths through life

A butterfly
which you want to keep with you forever
but at a certain moment
she flies away again
to continue her journey.

When a child with cancer sickens and as relapses become more frequent, the child understands that it is going to die (Bluebond-Langner, 1980). It is not always able or willing to speak about its approaching death, however. Parents and children may also try to protect each other (or themselves) against the pain and the sadness that talking about dying brings by remaining silent.
Sometimes a child uses symbols to show what is going on in its mind. Symbols for death and dying can be used as a guide to talk with the sick child, as well as with its siblings. The symbols should be part of the child's life experiences. In addition to the butterfly, dragonflies, fairy-tales, songs and characters from story and film can supply such symbols.
A number of symbols and ways to use them will be presented on the poster.

P-307

DEVELOPING THE NURSING CASE MANAGER IN THE SUPPORT ORGANIZATION

Sachiko Tahara . RN . School of health science. Tokai University.
Teruko Watanabe. RN. Kitasato University Hospital. Kanagawa, Japan

Although cancer is a malignant disease, we are now able to save the lives of more than half of the children who suffer from it. In the initial stages of the illness, the children must undergo relentless treatment. Even after the disease itself has been overcome, there was still the psychological harm inflicted on the child that must be considered. In 1978, we established the support organization in Tokyo, "Family Agency", in order to provide opportunities for the children to have fun and lively interaction with each other and to give them the courage to overcome their illness and also feel the joy of being alive. Our activities are summer camp, Christmas party, family session and counselling. The objects were accomplished.
Current focus of the nurse's activities in this organization is the use of primary care providers for children and their families in the terminal stage. We accepted some patients who were in the terminal stage. We evaluated the effectiveness of the nurses as a primary nurse .
This presentation will describe the role of nurse. The role of the nurse was to assess, monitor, plan and coordinate for the home care .
Especially, the effectiveness was evaluated based on the nursing staff of the hospital and parents satisfaction. What has been resulted is a new role in this support organization.

P-308

DAILY NURSING EVALUATION PAEDIATRIC ONCOLOGY

M.A. van Korlaar RN. Academic Medical Center (AMC), University of Amsterdam, Emma Children's Hospital, Department of Paediatric Oncology, PO Box 22700, 1100 DE Amsterdam, The Netherlands.

Since four years I work as headnurse in the paediatric oncology ward (21 beds, 30 nurses) of the Emma Children's Hospital within the AMC (teaching university hospital with 1,050 beds). Being engaged in the nursing of children with cancer since 1985, I became aware of the difficulties nurses face to keep their work and private life separated. Often they become too much involved with their patients. This asks for a clear distinction between the two subjects to avoid the burn-out syndrome.

To enable the nurses to disconnect from their work before going home, we initiated a daily evaluation schedule of half an hour where the nurses from the day shift discuss the problems of that particular day. Soon we realized that the structure was not very clear. At the same time there was a new development in the AMC (the external intercollegial quality check) which every ward had to follow. For this study we formed a working group and chose the daily evaluation as subject. So we could test whether the quality of the daily meetings met the goals. The three important items of this evaluation are: 1 reflection on our work, 2 relief of emotional pressures, 3 team work.

After nine months, the majority of the nursing staff claims to experience less psycho-social stress than before. Some aspects need further attention, for instance the fact that the nurses have to be present at a fixed time.

Conclusion, by implementing a daily nursing evaluation a burn-out syndrome can be prevented. Members from the nursing staff can better disengage themselves from the emotional aspects of their work, when given a chance to discuss these feelings. At the moment, we streamline these meetings for further implementation in other AMC wards.

P-309

"NUTRITION ADVICE FOLDER FOR FAMILIES".

Inger Westen Nurs, Liv Trogstad Enrolld nurse
and Kirsten Haugland Ward nurs, Pediatrichoncologi ward post 6,
Barneklinikken Rikshospitalet, Oslo Norge and
Lise Lotte Hoel Nursconsultant The Norwegian Cancer Soceiety,
Oslo.

Long term cytotoxisk treatment may cause reduced general condition and supplye of nutrition. Many children lose weigth and have poor apetite in this periode. The fact that parents and nurses want them to eat more makes them eat less. This children need to maintain a posetive relationships to food. We have asked ouer self : What can nurses do to solve this probleme?
We gathered all availeble information about nutrition and made these more avalible.
With: Education for staff twise a year. Routines for weight control oral vitamine. Orgenised all the offers from main kitchen. Nutrition advice folder availeble in kitchen and in parents kitchen and restroom. Each new familie get information how to use this folder.Desided when it is necessary to consider tube feeding.
Conlusion: We have been able to make our knowlegde know to the other staff and to families on the ward. Lather this year we hope to evaluate if this is tru. Do they know more, and is the information easy to find, and is the nutrition we offer the best we can manage for children in these circumstanses.
In this paper or poster I will show you the contents of this folder as an idea for others who are dealing with same probleme.

P-310

TRENDS OF RESEARCH IN PEDIATRIC ONCOLOGY NURSING IN JAPAN

Yoshiko KAJIYAMA, Yumi YOSHIDA and Mayumi INAGAKI
(College of Health Professions, Toho University, Tokyo, Japan)

Therapeutic treatments and diagnostic tests for malignant diseases have made remarkable progress in Japan in recent years. Many children have been saved and have come back to ordinary life after painful struggles, but they still have to experience real pain or boredom of rather long hospitalization, and not a few children and their families have to face a tormenting terminal stage.
Japanese nurses are trying hard to make hospitalized children happier and their lives healthier in their various dilemmas. To clarify nurses concerns, we looked into the research and reports done by Japanese nurses from 1989 to 1995. We referred to the Current Index to Japanese Nursing Literature(Japanese Nursing Association) and collected studies from the Journal of Japanese Pediatric Nursing, and Proceedings of the Society of Japanese Nursing Research. The methods of most articles were case studies, and investigations by questionnaires or interviews have been gradually increasing in recent years. The main themes were children's physical and psychological problems accompanying hospitalization and treatment, social problems such as returning to school, and care for terminally ill children and their families.

1

FOUR YEARS RESULTS OF TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN USING THERAPEUTIC PROTOCOLS WITHOUT PREVENTIVE CNS IRRADIATION

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Since September 1991 till December 195 have been treated 75 children with ALL according to two protocols: 53 (70%) pts with standard risk /SR ALL 0491/ and 22 (30%) pts with high risk /HR 0591/. There were 37 (49%) boys and 38 (51%) girls aging x 6,1years /0,5 - 15,7%y/. Multidrug therapy without preventive radiotherapy of neurocranium, consisted of VCR, Prednison, L asparaginase for SR and Adriamycin for HR respectively , in induction phase. During consolidation phase: HDMTX, 6 MP, Vumon, AraC, Cyclophosphamid and intrathecal MTX or triple drugs was applicated. The treatment lasted 24 and/or 30 months.
Complete remission /CR/ was obtained in 74 (98%) pts. One pt who did not achieve CR died on Pseudomonas septicemia. During CR we have lost pt on toxicity of therapy. 16children (21%) relapsed: 6 (8%) pts in bone marrow /BM/, 4 (5,3%) pts in CNS, 5 (6,6%) in BM + CNS, 1 (1,3%) pt in BM + testis. Overall EFS at 4 3/12 years was 62%, for SR groups 64%, for HR group 46%. Overall survival at 4 4/12 years was 82%, for SR group 88%, for HR group 65%.
The relative high rate of CNS involvement (9pts, 12%) in spite of HDMTX treatment indicates the need of this event, mainly HR ALL pts.

2

DESIGNING AND IMPLEMENTING A LARGE CLINICAL TRIAL IN CHILDHOOD A.L.L. ACCORDING TO A STRATEGY OF PROSPECTIVE META-ANALYSIS.

Valsecchi M.G., Silvestri D., Zimmermann M., Ochsenreiter K., Galimberti S., Suciu S., Meyer U.,Schuler D., Gadner H., Otten J., Conter V., Schrappe M., Riehm H. and Masera G. *for the I-BFM Study Group*.

Prospective meta-analysis (PMA) can be an useful methodological approach to the conduction of inter-group collaborative studies. According to PMA, different groups carry out simoultaneously treatment protocols that have the same randomised question but may be different otherwise. The principle underlying this approach is that a reliable answer to the randomised question can be obtained by pooling the data from each group. The most likely scenery is that the results from each group will point in the same direction (lack of qualitative interactions) and thus the study will provide an overall estimate of treatment effect. Heterogeneity of results could instead generate hypothesis on the role of the new treatment in different protocols.
Due to the results presently achieved, only moderate improvements, of the order of 5 to 10% increase in the event free survival (EFS), can be reasonably expected with new treatment schedules for the majority of children with acute lymphoblastic leukemia (ALL). Various European pediatric oncology groups (AIEOP, BFM, CLCG-EORTC, HUNGARY) have thus agreed on a large inter-group study on pulses during maintenance therapy for intermediate risk ALL patients, based on a strategy of PMA. The prospective nature of the study requires that a consensus is reached, prior to the beginning of the study, on major issues regarding the trial quality, allowing each group to keep its own internal organization for the conduction of the trial. The different groups have to: harmonize the specification and collection of the data relevant to the common randomized question; implement adequate randomization procedures and quality control measures; define the endpoints, time and methods of analysis and the modalities of creation of a pooled data base. With an expected 4-yrs recruitment of 1500 subjects, the power of the study (with α=0.05) will be 80% (or 90%) to detect a 6% (or 7%) difference, from 75% to 81% (or 82%) EFS at 4 yrs.
The PMA strategy for the conduction of a large trial requires good communication among groups but no heavy centralization of resources. It allows to conduct collaborative research prospectively on large samples without requiring treatment protocols to be exactly the same in all groups, but requiring nonetheless a standard level of treatment and study quality.

3

CYTOGENETIC ANALYSIS IN 55 CHILFREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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CHILDREN`sS HOSPITAL ZAGREB, ZAGREB, CROATIA

This paper presents the results of the cytogenetic analysis in 55 children with acute lymphatic leukemia (ALL). Acquired chromosome aberrations were identified in 35 (63,6%) children. Difference in frequency of clonal aberrations depending on age of patients was observed. Thus acquired aberrations were detected in all the three children up to 6 months of age, in 57,8% in the group from 1-1o years and in 6 (85,7%) out of 7 children older than 1o years at diagnosis. The analysis revealed chromosome aberrations specific for ALL. Hyperdiploidy > 5o was identified in 2o% of children, and the association with CALLA+ early pre-B and L2 - ALL was observed. Del(6) and 6(1;19) were identified in 7,3% and 5,5% of children. No association with a specific morphology or specific immunophenotype for both structural aberrations has been established. The association of structural aberrations involving regions 14q11-12 and T-cell ALL has, however, been observed since an aberration was identified in 2 (3,6%) children i.e. in 25% of our T cell leukemias. Interstitial deletion of the lond arm of chromosome 13, a rare chromosomal aberration in ALL, was identified in addition to del(9)(q31) in a 17 month old girl with constitutional trisomy of chromosome 21 and B-cell ALL-L2. Interesting is the finding of hyperdiploidy with 52 chromosomes and structural aberrations of chromosome No. 1 in one month old girl with morphologically unclassified CALLA+ pre-T acute leukemia. To our knowledge this is the first case of hyperdiploidy > 5o in a neonatal leukemia.

4

PROTOCOL YU 0187: A.L.L. TRIAL REPORT

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The Yugoslav co-operative group for hematology-ONCOLGY has existed over twenty years. After the breakup of the former Yugoslavia we in Serbia and Montenegro have continued its work. One of the important projects has been the production of protocols for treating A.L.L. The first protocol was YU0184 opened in May 1984 and closed in May 1987. The next protocol, YU0187, was opened in May 1987 and closed in May 1995. In Belgrade and Novi Sad, 127 children were entered. After the breakup of Yugoslavia, we have not received data on children included in these two protocols from Zagreb, Sarajevo, Skopje and Ljubljana. Both protocols were designed according to BFM protocols but stratification into risk groups were made under Children Cancer Group USA criteria. Remission rates in YU0187 was 94.18%, induction deaths were 4.2%, relapsis rates was 16.5% and five year disease free survival rates was 79.7%.YU0187 is better than the previous protocol with statistically significant improvements in induction death, percentage of relapsis and five year disease free survival. On the basis of data in references (Konja J., et al.; Ann of Oncol. 5.: Suppl. 8. 131. 1994) we have seen that the results in Zagreb (Croatia) are almost the same. We can conclude that the results of treating children with A.L.L. are good and that they are in line with the large national and international co-operative group results.

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RESULTS OF TREATMENT OF CHILDHOOD ANLL WITH BFM-87 PROTOCOL (IN MODIFICATION).

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Between May 1991 and January 1995, 32 consecutive children with acute nonlymphoblastic leukemia (ANLL) were treated according to the BFM-87 protocol excluding early intensification (high doses Ara-C and VP-16). The FAB-classification was M1 in 4, M2 in 16, M3 in 4, M4 in 4, M5 in 4 patients. The age was from 1 year 8 month to 15 years. The expression of both myeloid and lymphoid associated antygens was seen in 9 patients: T- and B-cells antigens were in 6 and 3 pts correspondingly. Of the 32 pts, 30 achieved a CR (90,6%) and 3-years EFS was 54,7%.
As compared with treatment results of "7+3" program, intensive program BFM-87 did not improve the EFS in: 1) females, 2) age <2 years, 3) WBC 20*10/l and more, 4) liver size more then 3 cm below the right costal margin, 5) M5 FAB, 6) expression of the B-cells associated antigens on the blast cells.
There is a need for more effective therapy with new agents for these patients.

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FAGOT FORMATION IN APL CELLS WITH CHLOROACETATE ESTERASE IN pH 8.0 BUFFER

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Acute promyelocytic leukemia (APL) is rare in childhood. Cytologically, two types are recognized: hypergranular and microgranular. The latter is far less frequent in children and it is disclosed with difficulty. The diagnosis is based on clinical, cytological, cytochemical and cytogenetic analyses. In pathological promyelocytes granules are better recognized when stained by Leishman's stain as opposed to standard MGG staining. Cytochemical chloroacetate esterase yields highly positive Auer rods against unstained background in fagot formations (according:Hayhoe F.G.J., and Quaglino D. 1980), which is the characteristic marker for APL, because no other form of pathological cells yields such a finding. Staining technique is simple and lasts 15 minutes only.
Eleven patients were evaluated for APL by using Leishman's staining and chloroacetate esterase in buffer pH 8.0, which was successful in confirming the diagnosis according to current protocols (Vesanoid). Cytogenetical findings obtained after a few days confirmed the diagnosis of APL. Chloroacetate esterase does not yield such remarkable findings in other pH buffers.

7

UMBILICAL CORD BLOOD AS THE SOURCE OF HEMOPOIETIC PROGENITORS FOR BONE MARROW TRANSPLANTATION.

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20 samples of human cord blood after term deliveries, 5 after pre-term deliveries and 10 samples of umbilical cord blood plasma were studied. Methods of clonogenic culture in methylcellulose (14 days) in the presence of Epo, rIL-3, rGM-CSF and in agar system (7 days) with normal human leukocytes as the source of growth factors were used.

Great number of hemopoietic progenitors (total - 11826,1+2894,6 in 1 mL. of umbilical cord blood) with prevalence of granulocyte and macrophage progenitors: 7880,2+2058,1 and 2706,8+642,1 in 1 mL of blood respectively were discovered in umbilical cord blood after term deliveries.

Clonogenic efficiency of CFU-GM after pre-term deliveries in agar system exceeds clonogenic efficiency after term deliveries in 1,5 times: 97,6+25,5 and 64,9+17,6 (x 10^5) respectively. Comparison of results of clonogenic culture in methylcellulose and agar leads to suggestion that these two cultural systems discover different GM-precursors.

These data proves that umbilical cord blood after term and pre-term deliveries contains great number of hemopoietic precursor cells and may be used as a source of stem cells for transplantation.

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TREATMENT OF CHILDREN WITH HIGH RISK NEUROBLASTOMA AND ACUTE LYMPHOCYTIC LEUKEMIA (ALL) AND NHL IN CZECH REPUBLIC.

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BMT Units at Univ.Hospital Motol in Prague serve as the only BMT centers for children in the Czech Republic. Practically all high risk neuroblastoma and non Hodgkin lymphoma pts and majority of high risk ALL pts are treated at the same hospital. Hematopoietic rescue with cryopreserved autologous grafts of BM or PBSC following the high-dose chemotherapy, surgery and/or irradi ation is a therapy of choice for high-risk neuroblastoma. Thirty five children (23 male and 12 female) with prognostically poor neuroblastoma,and no compatible related donor were entered into the study since early 1992. Entry criteria were stage, N-myc>10, ferritin, histopathology (Shimada classif) After 3-4 cycles of chemotherapy (CCG 3891) graft collection and processing yielded enough progenitor cells ($7.6x10^4$ (0.1-15.1 range) CFU-GM /kg b.w., 2.8×10^8 (1.3-4.4 range)/kg b.w. nucl. cells) and no evidence of clonogenic NB cells. Back-up unpurged grafts are also stored for most pts. Presence of clonogenic cancer cells necessitates ex vivo graft purging.We have utilized the known increased sensitivity of neuroblasts to iron deprivation to develop a "physiological" purging protocol. A 72-hour incubation with 50 uM chelator Deferoxamine results in log-5 decrease in neuroblasts,and no evidence of cluster forming activity; only log -.5-.9 decrease occurs in the CFU-GEMM cells. Eighteen children were transplanted so far; 16 had reconstituted{ANC>1K on day 18 (16-27), platelets>20K on day 27 (22-33)}; 2 died 12d and 21d post-transpl. (TRD); 5 relapsed and died; 9 are in CR after 4-42 months.

In a parallel study 22 children with poor-prognosis ALL and NHL (most after an early relapse), treated according BFM 90, BFM 90 REZ or "Lyon" protocol (Vanda),with no available BM donor were harvested for either BM or PBSC. Ex vivo purging protocol, using a 3 hour incubation with 25ug/ml VP-16 and 3 mg/ml methylprednisolone had previously been shown to result in a log 5-6 decrease in blast cells and only log 1-2 decrease in stem cells. The post-purge cryopreserved grafts of our patients contain 4,2 (2.1-6.3 range) x 10^8nucleated cells/kg b.w. ; 0.3 (0.0-1.0 range) x 10^4 CFU-GM/kg b.w. with no evidence of blasts.Ten of the patients were transplanted; all 10 were given G-CSF(5ug/kg daily) from day 6 until reconstitution{ANC> .5K; day 22 (15-34 range), platelets >20K on day 37 (24-50 range), 2 had prolonged thrombocytopenia}. Four are alive in CR (1-14m), 5 relapsed and died (BM relapse 3-11.5m), 1 died 10 d post transplant (TRD). (Supported by grants from Health Min.of Czech Republic).

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BONE TUMOURS IN CENTRAL SLOVAKIA - A RETROSPECTIVE STUDY

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INTRODUCTION: Osteosarcoma and Ewings sarcoma are the most common types of bone tumours in childhood.The aim of this study is a retrospective analysis of our patients, their treatment, follow up and causes of death.

PATIENTS AND METHODS : 579 children with cancer were treated between 1980 - 1995 in our clinic, 27 of them with bone tumours (4,6%). 13 males, 14 females, mean age 14 years (from 4 to 16 years). 11 patients (pts) had osteosarcoma (OS), 14 pts Ewings sarcoma (ES), 1 pt malignant fibrous histiocytoma (MFH), 1 pt malignant haemangiopericytoma (HPC). Benign bone tumours were excluded from the study. At diagnosis 26 pts had localised tumour to the primary site and 1 pt presented with lung metastases. 6/11 pts with OS had complete resection with endoprosthetic replacement, 4/11 amputation, 1 pt had only opened biopsy. 4/14 pts with ES had biopsy, 3/14 partial resection, 7/14 complete resection. Pt with MFH had complete resection. CT regimens were changed throughout the years.

RESULTS: 14 pts are alive (51,8%) - 7 with ES 6 with OS, 1 with HPC. 3 pts are still treated by CT.11 pts are free of disease after the end of the CT with median follow up 84 months (from 6 months to 10 years). 1 pt with OS had a second malignancy. 13 pts died (2 had local relapse, 8 developed distant metastases and 3 died because of toxicity of the treatment).

CONCLUSION: Bone tumours are rare in childhood, but their prognosis is still poor. In the last time survival can be improved by new therapeutic approach - aggressive CT with ABMT or PBSCT. This therapeutic method will be possible to use in our centre in the next year.

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TREATMENT RESULTS IN EWING'S SARCOMA

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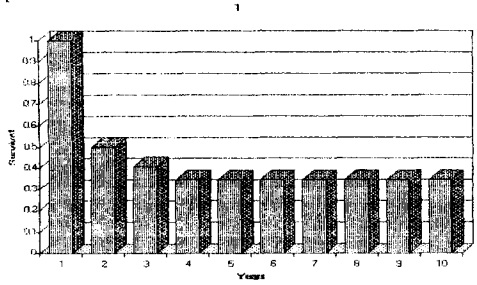
In the petiod 1981-95 years 32 patients with non metastatic Ewing sarcoma were traeted. Age distibutions were: upto 4 years-2 children; from 5 to 10 years-14 children, from 11 to 14 years-11 children and over 14 years-5 adolescens. From that group 85 % of patients were with locally advanced huge tumor (over 8 sm in diameter with infiltration in soft tissues). The primary tumors originated from: fibula-10 pts., femur-5 pts., pelvis-5 pts., humerus-4 pts., others-8 pts. In 8 pts. was made a radical surgery. The treatment of all patients was complex-radio and chemotherapy. In the tumor region the realizable local dose was 60-64 Gy(10 Gy for week). The chemotherapy was done according to protocol VACA(Vnc, Adria, Cyclo, Acti D). The survival rate analysis determined: 1 year survival-100%, 2 year survival-50%, 3 years survival-40%, 5 year survival-34%. Local tumor control was achieved in all patients with primary tumor localisation in long bones and only in 1 from 5 patients with pelvic localisation. In 2 cases (6,2%) local recurrence was observed on the 6 th and 8 th months after radiotherapy. The reported treatment results confirmed the systemic character of Ewing sarcoma and the necessity of complex treatment.



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JAMES EWING (1866-1943)

Arty R. Zantinga, Max J. Coppes. From the Tom Baker Cancer Centre, Alberta Children's Hospital and University of Calgary, Calgary, Alberta Canada.

Ewing graduated in 1891 from the College of Physicians and Surgeons of New York and subsequently, did his internships at the Roosevelt Hospital and Sloane Maternity (New York). He then became a histology instructor at Columbia University and in 1897 was promoted to Assistant in Clinical Pathology under Dr. Prudden, a man who would influence his life significantly. At age 33, Ewing was appointed the first Professor of Pathology at Cornell University, a post he would hold with great distinction for 33 years. In 1907, Ewing co-founded the American Association for Cancer Research, serving as its first president for two years. In 1913, he co-founded the American Society for the Control of Cancer (now The American Cancer Society). He also was a founding member of the Journal of Cancer Research. In 1919, after 7 years of incessant labor, Ewing's classic textbook "Neoplastic Diseases" was published and remained through four editions the standard reference for the pathology of tumors. This work, translated into several foreign languages, is a cornerstone of modern oncology. Ewing was convinced that cancer does not represent one disease but rather that the word *cancer* encompassed a variety of neoplasms. Consequently, his book presents the main features of origin, natural history and histopathology of the various tumors separately and stresses that the clinical course depends upon the histopathology. In 1920, Ewing described a malignant bone neoplasm which he initially designated '*endothelioma of bone*', because he believed it to arise from the blood vessels of the bone tissue; but in a subsequent report he admitted that the histopathologic picture was complex. This time he referred to it as '*endothelial myeloma*'. Today, this tumor is still called "Ewing sarcoma". Ewing was among the few who early on recognized the potential of radiation treatment of cancer. His interest in radiotherapy led him into considerations of radiation physics and radiobiology. Ewing died at the age of 76 years. He was a no-nonsense person, who despite his many contributions into the field of oncology, remained a humble man.

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OSTEOSARCOMA : A RETROSPECTIVE STUDY

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From 1981 to 1994 twenty-five children with osteosarcoma were treated at our institution.There were 13 boys and 12 girls with a median age of 10 yrs (range 4 to 14).The primary tumor was located in femur 12,tibia 11 and humerus 2/ 25 patients (pts). All had localized disease at diagnosis. Local treatment after biopsy unterwent changes throughout the years. 7/25 pts who were treated till end of 1985 had primary amputation and received various adjuvant chemotherapy (CT) regimens with VCR, MTX, ADRIA and BCD. Six died with progressive disease within one year from diagnosis and one is cured. 18/25 pts were treated after 1986. 17/18 received preoperative CT with ADRIA-CISPLATIN and then limb salvage surgery with endoprosthesis 16/17 and secondary amputation 1/17. One child received the same regimen plus IFO when relapsed in the lung three years after primary amputation. Within the series of these 18 pts: SIX died (with progressive disease to the lung 4/6, to the CNS 1/6 and one from acute myeloid leukemia five years after the end of therapy). NINE pts (50%) are alive in 1st complete remission (CR) with a median follow up time of 54 mo (range 27-90) from diagnosis and ONE in 2nd CR after lung metastasis resections and chemotherapy with HDMTX, IFO, VP16. TWO pts recently had disease progression (local 1/2,lungs 2/2). In two children with severe infection at the site of the endoprosthesis the surgeons decided delayed amputation 2 and 5 years after the procedure. It is believed that aggressive pre- and postoperative CT and advances in techniques for limb salvage surgery should result in improvement of the prognosis in our pts.

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TEN YEAR EXPERIENCE WITH OSTEOSARCOMA AT EGE UNIVERSITY PEDIATRIC ONCOLOGY UNIT

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Between January 1986 and December 1995, 31 patients diagnosed as osteosarcoma at Ege University Pediatric Oncology Unit. Sixteen were male, 15 were female (Male /female ≅1). Their ages ranged between 4 and 18 (mean 14.2±3.2). On admission, 16 (52%) had local pain and swelling and 1 had pathological fracture. The distribition sites of primary tumours were distal femur in 21, tibia in 7 (6 proximal, 1 distal), ileum in 2 and proximal humerus in 1 patient. Eight had pulmonary metastasis at first screening while 23 were nonmetastatic. All underwent open or tru-cut biopsy. Three patients refused treatment shortly after the diagnosis. Of 28 patients, 6 had surgical extirpations of the tumour by amputation before chemotherapy (CT), 5 were performed amputation after CT (VP-16, CDDP, ADR, CYC), 16 were performed limb salvage surgery after CT (IFOS, CDDP, ADR, MTX) and 1 with iliac localization have not underwent surgery. In 20 patients the pathological response to CT was evaluated before surgery and was found 85-100 % in 5 patients. Twelve of nonmetastatic 20 patients suffered from pulmonary relapse. Two died of drug intoxication (HDMTX). Two year-overall survival (OS) is 43.7% and mean follow up time is 15.7 ± 3.6 months for metastatic patients (n=8), two survived more than 24 months. Two year-OS is 51.1%, 5 year-OS is 27.2% and mean follow up time is 31.91 ± 6.58 months for nonmetastatic patients. Of all, 2 year-OS is 49.5%, 5 year-OS is 22.6%.

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RESULTS OF TREATMENT OF OSTEOSARCOMA IN 20 CHILDREN

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In the period from 1990-1995 there were 20 children with diagnosis osteosarcoma treated at the Children's Hospital Zagreb- Center for solid tumours. There were 10 boys and 10 girls aged 6-18 yrs in the examined group. Diagnosis was defined in children by one of the cytomorphological methods and confirmed by pathohistological analysis. According to TNM classification before treatment 19 patients (95%) were classified as T2N0M0 and in one patient metastatic changes were found (T2N0M1). Eighteen patients were treated preoperatively with HD MTX (Rosen T-10 protocol or Delepine modification) while in 2 patients surgical treatment was performed immediatly after diagnosis without preoperative chemotherapy. After the preoperative chemotherapy T1N0M0 was found in 14 patients (65%). In 13 patients partial bone resection was done (3 death), and in 7 patients limb amputation (3 death). In 7 patients treatment was started within one month of diagnosis and in 13 patients treatment started later (2-6 months) after the first symptoms. After the preoperative chemotherapy T1N0M0 was found in 14 patients (65%). Based on postoperative pathohistological finding (according to the % of necrosis) chemotherapy by protocol T-10 or its modification was continued in 16 patients, while 4 patients received CDDP+Adr. In 6 patients we found metastases: in 1 patient at the time of diagnosis while 5 of them developed metastases after treatment. Patients with metastases received as second-line chemotherapy: Holoxan, Carboplatina, VP-16 and Cis-platina with *radiation. A total of 6 patients died (5 patients with metastatic progression of* disease, 1 patient- complication of sepsis).
The aim of the study was examine success of treatment of osteosarcoma in correlation with: start of treatment, finding of metastases, increased value of alkal phosphatasis and LDH and scintigraphic finding. The results are shown in the study. In this study the results of complication are also shown.
Fourteen patients are intensively followed up. In conclusion: we have worse results in patient with spreading of the tumour into soft tissue, despite preoperative chemotherapy, in patients with increased values of alkal phosphatasis and LDH and in patients without improvement of scintigraphic finding after preoperative chemotherapy. There is question arising from our experience for additional therapy in such cases.

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HIGH DOSE IFOSFAMIDE IN MULTIFOCAL OSTEOSARCOMA: NEW APPROACH TO BETTER QUALITY OF LIFE.

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Multifocal osteosarcoma (M.O.) is rare entity and uniformly fatal. From 09/88 to 02/96,6 (PTS) with MO were treated. There were 4 males and 2 females (median age: 13 years, range: 4 - 16 years) primary site was femur in 5 patients and short time of evolution (40 days). Serum lactic dehydrogenase was elevated in 4 pts (mean: 463 IU, range: 184 - 897 IU) as well serum alkaline phosphatase (mean: 1892 mg%, range: 190 - 4388 mg%). Two patients received high dose methotrexate (12 g/sqm) dying with progressive disease two months (m) after the diagnosis. One patient received 5 courses of ifosfamide (IFOS) (1,8 g/sqm/day x 5 with mesna), adriamycin (ADR) (25 mg/sqm/d x 3) and cisplatin (CDP) (120 mg/sqm/d) with stable disease and improvement of the pain for 4 m. Three pts received two courses of a continuous infusion regimen of high dose ifosfamide (3 g/sqm/day x 5 with mesna) and after that they were treated with alternating courses of IFOS/ADR and CDP/ADR. All of them achieved partial response and a good quality of life for 8,11 and 13 m.
Further studies are necessary to determine the role of high dose chemotherapy to achieve better survival for these patients.

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THE RESULTS OF TREATMENT OF BONE TUMOUR IN CHILDREN AND ADOLESCENTS

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Between I988 and I996 28 patients(pts)with age range 4-I8years were diagnosed as having osteosarcoma of femoral bone-I9,tibial-5,brachial-3, cranium-I.In 6 cases there were lung metastases at diagnosis,5-had pathologic fractures in the tumour focus,2pts were considered incurable due to their condition.In 5 cases parents refused from any treatment.Ipt left for treatment in another region.I9pts with osteosarcoma were treated at Oncology Department of N.Novgorod Paediatric Clinic.The treatment included preoperative chemotherapy,surgery,postoperative chemotherapy. Preoperative chemotherapy consisted of 4 courses of adreablastin-30mm/m-on days:I,2,3 and cisplatin on 4th day intravenously with the interval of 3 weeks.There were done 3 limbsaving operations and I6 amputations.Histologic response asses ment determined the postoperative chemotherapy. III-IV-grade osteosarcona pts followed the protocol of preoperative chemotherapy,while I-II-grade cases received venesit and methotraxate. The IInd group included I9pts:4-are being treated,4-died due to lung metastases within 4-I2 months after the operation(amputations-3,limbsaving-I),II were discharged having survived the operation for Iyr-2,2yrs-4,3yrs-2, 4yrs-2, 5yrs-I. To improve the survival rate and life-quality of the patients chemotherapy is to be intensified to increase the amount of limbsaving operations that are not always possible in the existing clinical conditions.

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NATURAL KILLER CELL ACTIVITY IN LEVAMISOLE TREATED CHILDREN WITH BRAIN TUMOR

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Levamisole (Le) as immunostimulating drug is widely used in therapy of adult's malignant tumors. Its use in therapy of children's solid tumors is not common and there is no data about Le application in therapy of brain tumor (BT) in children. Since the NK cells represent first defense mechanism against development and spreading of malignant tumors the aim of this study was to investigate influence of Le therapy on NK cell activity in children with BT. PATIENTS AND METHODS: Twenty seven patients (pts) aged 2-14 years , treated with irradiation and chemotherapy, entered this study. Ten out of 27 pts received Le in doses of 2,5 mg/kg body weight per os for three consecutive days every two weeks for 6-12 months (mo). The NK cell activity was measured by 51-Cr release assay using K-562 cell line as target cells. The number of CD 16+ cells was determined by indirect immunofluorescence with OK-NK monoclonal antibodies. RESULTS: The NK activity and number of CD 16+ cells were significantly decreased in children with BT. Depression was much more pronounced in those children who developed recurrences and metastases during period of follow up (24 mo). Therapy with Le significantly augments the number ($p<0,01$) and activity ($p<0,01$) of NK cells (Table). The incidence of intercurrent infections in Le treated pts was much lower (30%). CONCLUSION: Le used as an adjuvant drug during chemotherapy period stimulate immunoresponse in immunocompromised children with BT.

Le treated pts	**No**		**Yes**	
NK cell activity	N	x	N	x
Effector:target cells				
12,5:1	17	6,25	10	10,63
25,0:1	17	11,28	10	18,73
50,0:1	17	15,24	10	24,40
CD 16+proportion	17	0,05	10	0,08
number$\times 10^9$/L	17	0,13	10	0,24

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THE EFFECT OF G-CSF ON NEUTROPENIA CAUSED BY INTENSIVE CYTOSTATIC TREATMENT IN CHILDREN

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We studied the effect of G-CSF on bone marrow depression and on delay in starting next treatment after intensive cytostatic courses with known bone marrow depression.
Methods: From december 1992 till december 1995 174 intensive cytostatic courses were delivered to 39 patients, aged 0.7-16 years, with solid tumors, lymphomas or relapsed leukaemia. G-CSF (Neupogen) was given 5 microgram/kg/day, sc. or iv. during 10 days, starting 24-36 hours after the last cytostatic dose.
Results: In 174 courses with the addition of G-CSF we found 101 courses with granulocytopenia $\leq 1.0 \times 10E^9/l$. The mean number of days (±SD) below 1.0 was 7±2 days (range 1-11). Recovery of granulocytes >0.1 occurred at a mean of 7±3 days after the start of the G-CSF, >0.5 at day 8±3 and >1.0 at day 9±4. On 38 occasions proven infections occurred during granulocytopenia <0.5. Nineteen patients had to be readmitted to the hospital, 19 other had to stay longer. All received antibiotic treatment. 144 of the 174 courses were followed by other courses. Of these 144 courses 17 could not be given in time: 13 were postponed for 1 week, 2 for 2 weeks and 1 for 3 weeks. The reasons for delay were 3x granulocytopenia, 8x thrombocytopenia, 6x clinical.
As side-effects we noticed 13x bone pain, 8x muscular pain, 10x bone and muscular pain.
Mucositis WHO grade 0 occurred 160x, grade 1 3x, grade 2 5x, grade 3 5x, grade 4 1x. 3, 1x 4.
Conclusion: administration of G-CSF prevented granulocytopenia in 73 (42%) courses. Only 17 courses had to be postponed. Severe mucositis occurred on 6 occasions; other known side effects were seen in 18% of courses.

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CYTOGENETIC INVESTIGATION OF HEMATOLOGICAL DISORDERS IN CHILDREN FROM KIEV REGION

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Abstract:
Karyotyping of the leukemic cells were carried out at the Kiev Research Institute of Hematology and Blood Transfusion under support of the cytogenetic laboratory at the Children's University Hospital in Giessen (Germany, chief Prof. Dr. F. Lampert), which kindly have presented equipments, reagents, special literature and have helped to study our specialist. The bone marrow or peripheral blood samples were sent from the hematological department of Kiev Municipal Children's Hospital and the oncohematological department of Kiev Region Hospital. From 1992 to 1995 in our laboratory were performed successfully 58 cases with AL, 5 - MDS, 8 - CML, 6 - Non-Hodgkin lymphoma. The results of this investigations were presented in march 1994 on oncocytogenetic seminar in Giessen and in february 1995 on International Symposium "Acute Leukemias VI. Prognostic Factors and Treatment Strategies" (Münster). In 1995 we received data about higher frequency of cytogenetic markers of secondary and hybrid AL; among patients from Kiev region: 1/3 of children with ALL and 1/2 with AML had -5/5q-, -7/7q-, 3q-abnormalities, marker chromosomes and polyploidy, every third patient had bi- or three abnormal clones. In addition in 4 from 27 cases of AL we registrated the evolution of leukemic clone before treatment. These data are very useful for clinicians to optimizade therapy regimes and witness about necessity to continue cytogenetic investigation among hematological patients for reveal cytogenetic peculiarities in ecological situation in our region.

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TREATMENT WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR RELAPSED HEPATOBLASTOMA

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The prognosis of relapsed hepatoblastoma(HB) is dismal, unless the tumor is resected. Since May 1989, myeloablative therapy (MAT) followed by autologous bone marrow transplantation (ABMT) has been applied to the treatment for relapsed HB. The purpose of this report is to investigate the effect of MAT and ABMT on the prognosis of patients with relapsed HB. METHODS: Five patients aged from 8 months to 4 years 2 months with relapsed HB were treated with MAT and ABMT. All patients received initial treatment with preoperative chemotherapy consisting of CDDP and THP-ADR and subsequent complete resection of the residual liver tumor. Two patients, who had relapsed tumor in the liver or multiple pulmonary metastases postoperatively, received MAT and ABMT after the normalization of alpha-fetoprotein (AFP) by the treatment with chemotherapy and surgical resection of the pulmonary disease. In other 3 patients, the elevation of AFP occurred despite postoperative chemotherapy consisting of CDDP and THP-ADR, whereas recurrent tumor was not detected on imaging examination. The MAT regimen consisted of melphalan and Thiotepa. RESULTS: In the patient with liver disease, tumor recurred in other site of the liver 1 year after ABMT despite the complete remission(CR) of first relapsed tumor. This patient received surgery and chemotherapy subsequently, and is CR 5 years from the last operation. In the patient with pulmonary metastases, tumor recurred in the brain and mediastinum, and died 9 months after ABMT. Three patients who presented the elevation of AFP alone are CR between 16 months and 4 years after ABMT. CONCLUSIONS: Treatment with MAT and ABMT for relapsed hepatoblastoma is effective on the cases with the elevation of AFP alone. However, it was not sufficient to achieve CR for the cases with relapsed tumor apparently on imaging examination.

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THERAPEUTICAL ASPECTS IN CHILDREN'S HODGKIN LYMPHOMA

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Purpose: to determine the therapeutical answer, survival and life quality on the basis of clinical and hystological criteria for Hodgkin lymphoma children.

Material and methods: Hodgkin disease represent 11% of our casuistry covering a 10 year perioud (1984-1993). Out of 86 cases, we studied 73 patients, medium age 10,08, 43 boys and 30 girls with different clinical stages with a II A predominance with hystological types according to Lukes-Butler's classification.

The therapeutical attitude was chem otherapy (COPP/ABUD) differently associated to radiotherapy.

Results: It was revealed on the studied batch a global survival of 74,9% at 5 years with a significant difference at stages II compared to stages IV (p=0,004) and for the favorable histology (lymphocyte predominance, nodular sclerosis) compared to unfavorable histology (mixed cellularity, lymphocyte depletion) p=0,037.

Therapeutical answer
- CR = 64,38%
- PR = 19,18%
- EV = 16,44%

The perioud free of disease at cases in CR at 5 years was 92%

Conclusions: Our results on non-selected batch are comparable to existing literature data concerning the survival and help in combining the results in prognostic groups. The comparatively good results obtained in stages III and IV are explained by the association of an aggressive chem otherapy of ABUD type to radiotherapy. The majority of therapeutical failures was generated by the extension of the disease in non-irradiated volumes, underlining the neccessity to associate a more aggressive chemotherapy.

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TOTAL TUMOR BURDEN(TTB) AND AGE AS THE MOST IMPORTANT PROGNOSTIC FACTORS IN CHIDHOOD HODGKIN'S DISEASE(HD) STAGE II TREATED WITH CHEMOTHERAPY(CT) and INVOLVED-FIELD RADIOTHERAPY (IF-RT)

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From 1988 to 1992, 87 children with **HD** in stage II were undergoing initial treatment with MVPP and/or B-DOPA combined with **IF-RT** (29 Gy). The observation was ended on December 31, 1995. The first remission achieved all pts. Relapses occurred in 8 cases. The effect of 17 variuos prognostic factors (e.g.:age,systemic symptoms, bulky disease,number of anatomical regions present, mediastinal mass,**ESR**,as well as **TTB**) on the **RFS** were tested by the Cox regression model. For evaluation of **TTB** we used a method developed by Specht and al.(Cancer 61:1719,1988). The **TTB** grade over 8 (p=0.03), and the age above 11 years (p=0.06) were the determining factors predicting a higher risk of first relapse. We separated two risk groups: high risk group (**HRG**) with **TTB** grade > 8, and age > 11 years; low risk Group (**LRG**) with **TTB** grade <= 8, and age <= 11 years. The 5-year **RFS** rates were 76% and 98%, respectively for **HRG** and **LRG**. So, **HRG** pts needed a change of their treatment to more intensive. **CT** combined with **IF-RT** seems now to be the optimal method of treatment for **HD**. Intensity of therapy should be tailored to the stage, tumor burden, and age. There still is a need to determine new risk groups which require more effective treatment. Further modifications of treatment should improve the cure rate, while minimizing the complications.

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ACUTE NEPHROTIC SYNDROME AND HODGKIN'S DISEASE IN CHILDREN: A REPORT OF FIVE CASES

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This report documents the occurrence of the nephrotic syndrome in five children with Hodgkin's disease. In two cases the nephrotic syndrome predated the diagnosis of lymphoma by 6 months and 12 months, while in the other three, the two occurred simultaneously. The nephrotic syndrome resolved in four cases during effective treatment for active Hodgkin's disease, while proteinuria remained unchanged in the fifth case despite partial control of the lymphoma. The occurrence of an acute nephrotic syndrome as a manifestation of active Hodgkin's disease points to a role of lymphokine-mediated nephrotoxicity. The possibility of an underlying malignancy must be borne in mind when a nephrotic syndrome occurs in children with no other apparent cause.

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FOLLOW-UP STUDIES IN LONG-TERM SURVIVORS OF WILMS' TUMOUR

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Late effects of the illness and/or the treatment were studies in 27 children cured of Wilms tumour with a survival time of 2,4- 22,5 years (mean: 11,3 years) since the diagnosis. After the informed consent of parents and/or the former patients the investigation consisted of physical examination, anthropometria, laboratory examinations, X-ray and ultrasound investigations. As a result, we found that all children were in good general condition without any complaints or symptoms. They everyday activities, achievements do not differ from their normal peers. Their growth and development measured by the comparison of boneage to chronological age and by the standard deviation scores of height and weight were between normal limits. Some compensatory renal hypertrophy was found in all patients, but elevated blood pressure was not observed in anyone of them. No signs of drug-induced cardiotoxicity were seen with echocardiography. While some patients, who received radiotherapy have had scoliosis of moderate severity, as a well known side-effect of irradiation, only one patient needed orthopedic treatment for this disorder. No obvious pathological value was found during the laboratory investigation. However, the study of renal function gave some findings, which may indicate slight to moderate damage of glomerular and/or tubular function in some children. Summing up the results of our study we can state that the quality of the long term survival of children who were treated for Wilms' tumour is good, nevertheless, because of the possibility of any chronic, may be subclinical, late effects, especially in renal functions, further and repeated follow-up investigations are needed.

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DELAYED ADVERSE SEQUELAE OF CANCER THERAPY IN ADOLESCENTS

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Aim of our study was to evaluate adverse delayed effects in a subgroup of cancer patients—adolescents. Thirty-three patients entered the study (19 males, 14 females, at ages ranging from 13 to 21 yrs). They were affected with: CNS tumors (15%), Wilms tumor (24%), neuroblastoma (18%), histiocytosis (12%), Hodgkin lymphoma (10%), non-Hodgkin lymphoma (9%), sarcoma (9%), and hepatoblastoma (3%). They all underwent surgery (87.5%), radiotherapy (60.3%), and chemotherapy (96.8%), and they have been off-therapy for 3 to 19 yrs (mean: 8.18 yrs). We evaluated: heart, kidney, liver, gut, gonadal, neurologic and thyroid function, visual and hearing impairment, growth abnormalities, psychosocial development and second malignancies. All pts receiving radiotherapy showed a significant reduction in linear growth (2.6 cm; $p<0.05$). The reduction was more significant in pts receiving craniospinal irradiation (10.3 cm). Obesity was revealed in 18.2%; scoliosis in 63.6% (39.3% underwent spinal irradiation, 36.3% abdominal or chest surgery, and 33.3% had both), and 19% of cases required orthopedic treatment. Cardiac assessment showed an abnormal LVFS in 28%, with no apparent clinical signs. No significant impairement of renal function was diagnosed. Sexual development and hormonal values were not affected by treatment. Ages at menarche were normal. In 21% of males, FSH values were increased, but all these had normal testosterone blood values. Thyroid function impairment was found in 6%. Abdominal and chest radiotherapy after 6-8 years caused esophageal stenosis in 3 cases (9%) which required surgical dilatation. Object Relation Form (ORF) and Raven tests were used to investigate psychosocial function. All had a normal I.Q. (range 80-129), but in CNS pts it was slightly lower in range (99-105). The ORF revealed an adequate capacity in maintaining relationships in 75.8%, whereas in 24.2% there was some difficulty. Defensive-adaptative dynamics were mild in 37.9%, moderate in 27.5%, severe in 31%, and absent in 3.6%. Strabismus was diagnosed in 12%; one pt treated with aracytin revealed corneal opacities. Audiometric evaluation showed neurosensitive loss in 17%, transmission loss in 15%, and mixed loss in 43.5%; in pts affected with CNS tumors the mean loss was significantly higher (range 20-60 dB at 11,000 Hz). A second malignancy was reported in 2 girls. One girl with hepatoblastoma diagnosed at 3 yrs of age developed papillary thyroid carcinoma 10 yrs later and was treated with surgery and radiotherapy. A second girl with neuroblastoma diagnosed at 11 mos of age presented with benign pheochromocytoma 14 years later.

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SENSITIVE AND NON INVASIVE EVALUATION OF CHRONIC AND ACUTE CARDIOTOXICITY INDUCED BY ANTHRACYCLINE IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA

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Aim: We evaluated a series of pediatric patients with acute leukemia treated with anthracycline(ATC) to determine which variables of ECG and echocardiography might be sensitive indices for detecting chronic cumulative cardiotoxicity and acute cardiotoxicity of ATCs.

Patients and method: 33 ALL patients and 11 ANLL patients receiving ATCs such as Doxorubicin, Daunorubicin, Aclarubicin, Pirarubicin, and Mitoxantrone were eligible for this investigation. The cumulative doses of ATCs were 60 to 810 mg/m^2 in ALL patients, and 180 to 1555 mg/m^2 in ANLL patients, respectively. ECG and echocardiography were performed during and after chemotherapy.

Results: Regarding the chronic cumulative cardiotoxicity, only corrected QT time on ECG showed significant prolongation in the patients receiving over 700 mg/m^2 of ATCs. Endsystolic wall stress/endsystolic volume index(ESS/ESVI), indicating left ventricular contraction on echocardiography, had negative correlation to the cumulative doses of ATCs, and showed significantly low levels in many cases receiving 300 mg/m^2 or more, and in all cases receiving 500 mg/m^2 or more. Other variables (shortening fraction, left ventricular pre ejection period/left ventricular ejection time, and % of velocity of circumferential fiber shortening) also had correlation to the cumulative doses, and showed significant changes in the cases receiving over 700 mg/m^2. In the patients managed with intensified chemotherapy, ESS/ESVI of post-intensified chemotherapy was significantly lower than that of pre-intensified chemotherapy. Since this decrease was transient and reversible, we thought this change as acute cardiotoxicity of ATCs. This phenomenon was typical when the cumulative doses reached over 500 mg/m^2.

Conclusion: These results indicated that ESS/ESVI was sensitive index for detecting the cardiotoxicity induced by ATCs. Careful evaluation with echocardiography is recommended when the doses of ATCs reach 300 mg/m^2.

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IS THERE A ROLE FOR INTRAOPERATIVE RADIOTHERAPY (IORT) IN NEUROBLASTOMA ?

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Poor prognosis of advanced neuroblastoma is partly due to failure of local tumor control. Intraoperative radiotherapy (IORT) can promote locoregional tumor control in combined modality treatment by delivering a single large dose of radiation to the tumor bed at time of surgery.

We report 4 patients with advanced, initially unresectable neuroblastoma. IORT was performed using high energy electrons at 6-8 MeV at a dose of 8-10 Gy. Indication for IORT was residual tumor tissue at 2nd look surgery. Chemotherapy and postoperative additional conventional radiation therapy of 14-27 Gy was applied according to the GPOH protocol.

In all cases, IORT could be carried out without technical difficulties. Local tumor control was achieved in 3 of 4 patients (75%). Of the three responders, two children with stage 4 disease are in complete remission 15 months after IORT. In the third patient responding to IORT, postoperative recovery was complicated by mesenteric infarction requiring repeated surgical interventions. The child eventually died of septicaemia. One patient had local and systemic tumor progression during postoperative chemotherapy and died.

Electron beam therapy can be efficiently delivered to incompletely resected neuroblastoma at surgical exploration. The therapeutic ratio of local control to tissue toxicity is enhanced. Thus, an increased total radiation dosage can be delivered to the tumor bed by the combination of IORT and external irradiation compared to conventional radiation treatment alone. Since resectability of the primary tumor is one of the four-most relevant risk factors in neuroblastoma, IORT may improve outcome in patients with incompletely resected tumors.

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THE USEFULNESS OF MRI OF THE LOWER EXTREMITIES FOR STAGING NEUROBLASTOMA AND ESTIMATING THERAPEUTIC EFFECTS

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This study evaluated the role of MRI in investigating bone marrow metastasis(BMmet), staging neuroblastoma(NB) and estimating therapeutic effects. Using MRI, fifty-eight patients were investigated for BMmet of NB at our institute and associated hospitals between June 1986 and June 1995. Cytodiagnosis from bilateral iliac spines and MRI of the lower extremities were used to investigate BMmet of NB. Thirty of 58 patients were shown to have BMmet by cytodiagnosis or MRI. Twenty-nine(97%) and 19(63%)patients were diagnosed with BMmet by MRI or cytodiagnosis, respectively. In eleven patients(37%) diagnosed by MRI, BMmet was not detected by cytodiagnosis. Three(10%) of these 11 patients showed only BMmet without any other metastases and comprised one stage IV S and two stage IV NB patients (Evans staging). Without MRI findings, these patients would have been diagnosed to be one stage II and two stage III NB patients. There is a possibility that small number of NB patients with BMmet detected by MRI would have been included in early stage NB diagnosed by conventional methods. The residual rate of BMmet after chemotherapy was 53%(31/58) in 58 involved bones in 29 patients detected by MRI. Nine of 29 patients(31%) had BMmet detected by MRI only, and would have been diagnosed in complete remission by conventional diagnostic methods. It was shown that there are patients with BMmet that can only be detected by MRI of lower extremities ,and that accurate staging of NB as well as estimation of therapeutic effects cannot be achieved by conventional methods. Therefore, MRI is necessary for investigating BMmet of NB along with cytodiagnosis.

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Effect of retinoic acid on levels of calbindin D-28k and S-100 protein B in human neuroblastoma cell lines.

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Retinoic acid (RA) is known to promote morphologic differentiation of human neuroblastoma cell lines. The present study tests the relations between morphological differentiation and the expressions of Calbindin D-28k (calbindin-D) and S-100 protein B (S-100b) in 5 human neuroblastoma cell lines (GOTO, IMR, LA-N-5, NB-1, SK-N-SH). Calbindin-D and S-100 protein B are calcium-binding proteins that are found mainly in the nervous system. Our syudy showed levels of these two proteins in neuroblastoma tissues are correltated with prognosis of patients with neuroblastoma. Levels of calbindin-D and S-100b were measured by immunoassay. These levels were widely distributed in 5 cell lines (calbindin-D; 0.3 - 16.2 ng /mg protein, S-100b; 141.0 - 2560 ng/mg protein). RA induced increasing level of calbindin-D in two (LA-N-5 ;2.8 to 21.0 and SK-N-SH;11.2 to 27.3 ng/mg protein) of 5 neuroblastoma cell lines. These findings suggest that Calbindin-D may be an useful marker of neuronal differentiation in neuroblastoma.

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CLINICAL COURSE OF CHILDREN WITH NON-HODGKIN'S LYMPHOMA WITH ATYPICAL FEATURES AT DIAGNOSIS

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Abstract

There were 84 children with newly diagnosed non- Hodgkin's lymphoma /NHL/ treated at our Depatment since 1987 till 1995. The diagnosis included histo- pathological examination of fixed and frozen material and pheenotyping assay. This routine procedure was satifactory for the majority of cases, but in some patients unusual features occured.

We distinguished two subgroups of these children:

A/-with atypical diagnostic features: rare primary site /liver, bone,gingival, gastric paravertebral/ and/or atypical phenotype - 10 children;

B/ with concurrent problems :immunodeficiency and/or congenital malformations- 8 patients.

The course of disease of these patients was analysed and compared with the rest of patients.

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CLONALITY OF CHILDHOOD ACUTE LEUKEMIA (AL) BY X-LINKED DNA POLIMERIZATION

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We studied 9 girls with AL (8 - AML, 1 - ALL) to determine the clonality status of their hematopoiesis. It was used HUMARA - PCR clonality assay to analyze DNA from bone marrow cells and from skin as a control of skewed lyonization for every case. 8 girls were heterozygous at the locus of androgen receptor gene (HUMARA), permitting X-linked clonality assay to be performed (88,8%). 5 patients were investigated at the manifestation of the disease, 3 of them were tested twice: in acute phase and repeatedly in remission. 2 girls (AML, M2; ALL, L1) were in relapse at the time of the study, 1 girl - in remission. In all our cases control tests with skin DNA demonstrated polyclonal methylation patterns, so there were no skewed lyonizations in our series. Methylation analysis of DNA from bone marrow leukemic cells in acute phase and relapse showed the presence of a single digested (unmethylated) fragment in all cases, suggesting clonal origin of the leukemic cell proliferation. In three cases methylation analysis of bone marrow cells was repeated in 1-6 month during the remission. Two of them demonstrated the change of the clonal patterns of the methylation to the polyclonal type. In the third case hematopoiesis in remission was clonal again, but with a different allelic pattern. Hematopoiesis investigated at the first time in a patient with remission of 2 mo duration appeared to be polyclonal. The results indicate that polyclonal hematopoiesis can be restored not in every case of the remission. The genotype of clonal hematopoiesis in remission may be changed, the clinical meaning of these effects is studying.

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PROGNOSIS OF SURVIVAL FOR VERY YOUNG CHILDREN WITH *MEDULLOBLASTOMA TREATED WITH CRANIOSPINAL IRRADIATION*

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It has been reported that very young children with medulloblastoma carry a worse prognosis compared to older children. We analysed the literature data in order to test this hypothesis.

Publications were included in the analysis if radiotherapeutic treatment consisted of craniospinal irradiation by megavoltage equipment and patients had a minimum follow-up that allowed the calculation of at least 5-year survival rates.

Out of 34 publications with 2736 evaluable patients 9 studies analysed survival according to age at diagnosis. Most studies used different cut-off levels, with a variation between 2 and 16 years. Although in 8/9 studies the reported 5-year survival rates were up to 28% lower for the younger patient group, the difference was statistically significant in only two. Predominant site of failure is the primary tumour region. In very young children treatment differs by a reduction of irradiation dose compared to standard treatment. Literature data suggests that a dose of less than 50 Gy to the posterior fossa significantly reduces survival.

The hypothesis that very young children with medulloblastoma carry a worse prognosis is generally accepted but based on few data. A similar prognosis might be achieved with the combination of postoperative chemotherapy and delayed craniospinal irradiation and a minimum dose of 50 Gy to the posterior fossa.

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Abstract withdrawn.

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CALCIFYING FIBROUS PSEUDOTUMOR. REPORT OF 2 CASES.

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The calcifying fibrous pseudotumor (CFP) is a rare fibrous neoplasm of the soft tissue. This lesion, recently described, has an uncertain pathogenetic relationship with myofibroblastic pseudotumors. Two cases of CFP are reported. **Case 1**: a 7 year old girl was admitted for a right neck mass. The US demonstrated a solid nodule (2x2 cm) behind the sternocleidomastoid muscle. After a FNAB, which was not diagnostic, a surgical biopsy was performed and the histology demonstrated CFP. A more radical exeresis was proposed but the parents refused and the girl was lost at FU. In 2 years the mass showed a progressive growth and the pt was admitted again. After a CTscan, which pointed out a cervical lesion (9x9 cm) reaching the superior mediastinum, the cervical mass was removed with macroscopical residuals.The parents refused a second operation (thoracotomy) to achieve the complete excision. The child is alive with stable disease at 8 months from the last procedure. **Case 2:** a 3 year old boy presented 2 months after an ankle trauma with 2 nodules on the left ankle and leg. A biopsy of the malleolar mass revealed a CFP. The following RMI showed multiple deeply localized nodules. The popliteal vessels and the Achilles tendon were involved. We decided to perform a partial resection because a complete excision would have been mutilating. The histology confirmed CFP. The child is alive with stable disease 15 months after diagnosis. Both cases were histologically carachterized by a circumscribed noncapsulated fibrous lesion with scarse (myo-)fibroblastic proliferation and a sparse inflammatory infiltrate of plasma cellsand lymphocytes, focally aggregated with germinal centers. Calcifications were abundant in case 2, few in case 1. In this latter case the lesion entrapped at the periphery few skeletal muscle cells and adipous tissue. **Comments:** CFP are usually benign fibrous lesions. Few cases are reported; the surgery seems to be the only treatment. Our first case demonstarates that CFP, when incompletely excised, may have a rather rapid progressive growth which makes a successful excision difficult. Our second case is the first documented multifocal lesion. At this point the long-term outcome of these pts is uncertain.

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TUMORS OF THE KIDNEY IN CHILDHOOD: PATIENTS TREATED ACCORDING TO THE INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY - NEPHROBLASTOMA CLINICAL TRIAL STUDY PROTOCOL FROM 1989 - 1995.

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Introduction: Tumours of the kidney in children are mostly congenital. Benign are often cysts, which need surgical treatment only if they are unilateral. Malignant are often: nephroblastoma (Wilms), clear cell sarcoma of the kidney, rhabdoide tumour of the kidney and they need complex diagnostic and therapeutic approach. Malignant tumour of the kidney are often found in combination with different malformation syndromes and/or congenital anomalies.
Material and methods: We represent our experience with eight patients treated at Clinical Hospital Split from 1989 - 1995. They were 3 females and 5 males from 0-5 years of age. The dominant symptom in the beginning was tumour mass isolated with /or without congenital anomaly. The stages of tumors were as follows: 4 in the stage I (tumor limited to the kidney, complete excision), 2 in the stage III (incomplete excision without hematogene metastasis) and one in the stage V (bilateral tumor of the kidney). Pathohistologycally we found 2 benign and 6 malignant tumors: 4 of the left kidney, 3 of the right and one bilateral. According to Stockholm Working Classification of Renal Tumors of Childhood (1994) there were:
1. low risk "favourable histology": 3 patient in the stage I.
2. intermediate risk "standard histology": 1 patient in the stage V (bilateral).
3. high risk "unfavourable histology ": 2 patient in the stage III
4. other tumors and lesions: 1 patient in the stage I (multilocular cyst) and 1 patient with polycystic tumor of kidney.
Results: In family pedigree data of these patients we have found informations about hereditary diseases, spontaneous abortions and stillborns. In one patient we found fenotypycal characteristic of Denis-Drash syndrome without chromosomal changes in 11p13 (WT1), and the other with Beckwith-Wiedemann syndrome and del 11p 153-152. All patients where treated according to proposition of S.I.O.P. Committee for Nephroblastoma. They are alive and without signs of the disease.
Conclusion : We would like to emphasize the importance of multidisciplinary approach for diagnosis and treatment of the tumours of the kidney in childhood.Our results are derived from successful activity of our Clinical Hospital in S. I. O. P.

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TREATMENT OF RECURRENCES AND METASTASES OF WILMS TUMOR

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22 patients with Wilms Tumor were treated at the Paediatric Surgical Clinic of Arkhangelsk Medical Academy from 1989 to 1994. In 3 children a recurrent tumor was found 10, 11 and 14 months after the operation. In 2 patients metastases of lungs and pleura were identified 9 and 11 months after surgery, one of them had metastases combined with recurrent tumor. The diagnosis of malignancy was confirmed cytologically in all the cases. To treat metastases and recurrent tumors chemotherapy with combination of VP-16, cyclophosphane and epirubicine was administered. Four children were operated on after 4 cycles of chemotherapy: removal of the recurrent tumor (2), of the metastasis in parietal pleura (1), of the metastasis in pleura and the recurrent tumor (1). Pathomorfosis (histologic changes) stage III-IV related to treatment were documented in all the cases. During the postoperative period 8-10 courses of chemotherapy were used. One of the patients with metastasis of the lung received only chemotherapy and X-ray. The period of follow up is 18-28 months since the end of the therapy.

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RECOMBINANT HUMAN ERYTHROPOIETIN IN CHEMOTHERAPY-INDUCED ANAEMIA OF CHILDREN WITH MALIGNANT SOLID TUMORS

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We present preliminary results of a prospective, randomized study, designed to investigate safety and efficacy of rHu-EPO in the prevention and treatment of chemotherapy-induced anemia in children with solid tumors. Twenty children (age 4-18 years) with histologically proven diagnosis undergoing cyclic combination chemotherapy were randomized either to control or to receive rHu-EPO at a dose of 150 U/kg/dose subcutaneusly 3 times/week for a minimum of 12 weeks or 3 chemotherapy cycles. Efficacy of rHu-EPO therapy was assessed by comparisons of hematocrit, hemoglobin levels and transfusion requirements over the study period in control and EPO groups, respectively. Of 15 evaluable patients, 8 were randomized to rHu-EPO and 7 to control. R-HuEPO-treated patients showed an increase in hematocrit over the first 8 weeks of therapy, with significantly higher mean hematocrit at week 8 compared to control patients (33.2$\pm$ 2.1 % versus 39.3$\pm$4.2 % in control and EPO groups, respectively, $p<0.05$). Similarly, significantly higher hemoglobin concentrations could be demonstrated in rHu-EPO group by week 8 (11.06$\pm$1.35 g/l versus 13.11$\pm$1.13 g/l in control and EPO groups, respectively, $p<0.05$), with higher precycle hemoglobin before chemotherapy cycle 3 (11.59$\pm$1.21 g/l versus 13.01$\pm$1.34 g/l in control and EPO groups, respectively, $p<0.05$) and cycle 4 (11.07$\pm$1.06 g/l versus 13.04$\pm$1.74 g/l in control and EPO groups, respectively, $p<0.05$) and higher midcycle hemoglobin between cycles 3-4 (8.73$\pm$1.92 g/l in control group, 11.00$\pm$1.16 g/l in EPO group, $p<0.05$). There was a trend towards the reduction of transfusion requirements during the 3rd month of therapy in rHu-EPO-treated patients (0.57 units of red blood cells/patient in control versus no transfusion at all in rHu-EPO group, NS). RHu-EPO treatment was well tolerated, no serious side effects were reported. Individual observations suggest improvements in quality of life during rHu-EPO therapy in children.

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A STUDY OF ONDANSETRON SYRUP IN THE TREATMENT OF CHEMOTHERAPY-INDUCED NAUSEA AND EMESIS IN PEDIATRIC CANCER PATIENTS

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Introduction: Ondansetron is a highly selective 5-HT_3 receptor antagonist, and its tablet and injection have been shown to be highly effective in preventing nausea and emesis due to chemotherapy. In this study, we evaluated antiemetic effects and safety of ondansetron syrup, the new dosage form of ondansetron, in pediatric patients receiving various anticancer drugs.

Methods: Ondansetron syrup 2.5mg/m^2 was administered orally 1-2 hours prior to cisplatin or non-platinum agents in 179 pediatric cancer patients. The patients received any of the following emetogenic agents: Cisplatin$\geqq$50mg/m^2 (iv), Cytarabine$\geqq$500mg/m^2 (iv) or $\geqq$20mg/m^2 (it), Ifosfamide$\geqq$2g/m^2 (iv), Cyclophosphamide$\geqq$1g/m^2 (iv), Methotrexate$\geqq$500mg/m^2 (iv) or $\geqq$10mg/m^2 (it), Actinomycin-D$\geqq$15mcg/kg/day (iv).

Results: Anti-emetic effects were evaluated in 138 cases (79 male and 59 female, 7 months to 16 years 0 month of age). In a 24h period after starting chemotherapy, 97 (70.3%) of those patients had $\leqq$2 emetic episodes (i.e. complete or major responses). Children receiving high-dose cisplatin ($\geqq$ 75mg/m^2) presented a more difficult anti-emetic challenge (27.8%; complete or major response) compared to patients receiving 50-74mg/m^2 cisplatin (62.5%) or non-platinum agents (77.7%). No major drug-related side effects were reported.

Conclusion: Ondansetron syrup 2.5mg/m^2 was well tolerated and effective in children receiving emetogenic chemotherapy. However, higher dose of ondansetron syrup or intravenous administration of ondansetron may be required for patients receiving high-dose cisplatin.

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CARDIAC CHANGES DUE TO CHEMOTHERAPY IN ACUTE CHILDHOOD LEUKEMIA.

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To Identify cardiotoxcity in leukemic patients receiving variable percentages of maximum cumulative dose of anthracycline, clinical, roentegenographic, E.C.G., echocardiographic assessment and determination of serum levels of L.D.H. , AST, CK, and CKMB enzymes were determined for 45 patients with ALL at diagnosis, after induction of remission and after 6 months of recycling chemotherapy. Patients were stratified according to the percentage of maximum cumulative dose of anthracycline received into 25, 50 and 100% . Cardiomegaly was a prominent feature in newly diagnosed patients, after induction of remission and after 6 weeks of maintenance therapy . Sinus tachycardia was present in all stages of therapy . Serum levels of total LDH and AST were higher in newly diagnosed patients and decreased after induction of remission . Total CK level was within normal at diagnosis to increase significantly after treatment. CKMB was within normal at diagnosis and increased significantly along the course of therapy . It was found to be the most sensitive parameter. It is concluded that acute leukemia affects the heart due to the process of the disease itself and secondary to the effect of chemotherapy. The changes were maximally observed in patients receiving 100% of the maximal cumulative dose of the drug. Monitoring cardiac structure and function should be performed periodicaly along the course of chemotherapy to detect early changes that might necessitate discontinuation of anthracyclines and shift to other drugs .